



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/12, 5/10, C07K 14/705, 16/28, G01N 33/53, C12Q 1/68, A61P 25/00		A1	(11) International Publication Number: WO 00/29577 (43) International Publication Date: 25 May 2000 (25.05.00)
(21) International Application Number: PCT/US99/26701 (22) International Filing Date: 12 November 1999 (12.11.99) (30) Priority Data: 60/108,322 13 November 1998 (13.11.98) US 60/114,078 28 December 1998 (28.12.98) US 09/258,633 26 February 1999 (26.02.99) US 09/421,134 19 October 1999 (19.10.99) US (71) Applicant: MILLENNIUM PHARMACEUTICALS, INC. [US/US]; 75 Sidney Street, Cambridge, MA 02139 (US). (72) Inventor: CURTIS, Rory, A., J.; 31 Constitution Drive, Southborough, MA 01772 (US). (74) Agents: MANDRAGOURAS, Amy, E.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US) et al.		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: NOVEL MEMBERS OF THE CAPSAICIN/VANILLOID RECEPTOR FAMILY OF PROTEINS AND USES THEREOF			
(57) Abstract <p>The invention provides isolated nucleic acids molecules, designated hVR-1, hVR-2, and rVR-2 nucleic acid molecules, which encode novel members of the Capsaicin/Vanilloid receptor family. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing hVR-1, hVR-2, and rVR-2 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which an hVR-1, hVR-2, and rVR-2 gene has been introduced or disrupted. The invention still further provides isolated hVR-1, hVR-2, and rVR-2 proteins, fusion proteins, antigenic peptides and anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.</p>			

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NOVEL MEMBERS OF THE CAPSAICIN/VANILLOID RECEPTOR FAMILY OF PROTEINS AND USES THEREOF

Background of the Invention

- 5 Pain is initiated when the peripheral terminals of a subgroup of sensory neurons are activated by noxious chemical, mechanical or thermal stimuli. These neurons, called nociceptors, transmit information regarding tissue damage to pain-processing centres in the spinal chord and brain (Fields, H.L. *Pain*, McGraw-Hill, New York, 1987). Nociceptors are characterized in part, by their sensitivity to capsaicin, a vanilloid-
10 containing compound, and a natural product of capsicum peppers that is the active ingredient of many "hot" and spicy foods. In mammals, exposure of nociceptor terminals to capsaicin leads initially to excitation of the neuron and the consequent perception of pain and local release of inflammatory mediators. With prolonged exposure, nociceptor terminals become insensitive to capsaicin, as well as to other
15 noxious stimuli (Szolcsanyi, J. in *Capsaicin in the Study of Pain* (ed. Wood, J.) 1-26 (Academic, London, 1993). This latter phenomenon of nociceptor desensitization underlies the seemingly paradoxical use of capsaicin as an analgesic agent in the treatment of painful disorders ranging from viral and diabetic neuropathies to rheumatoid arthritis (Campbell, E. in *Capsaicin and the Study of Pain* (ed. Wood, J.)
20 255-272 (Academic, London, 1993); Szallasi, A. *et al.* (1996) *Pain* 68, 195-208). Some of this decreased sensitivity to noxious stimuli may result from reversible changes in the nociceptor, but the long-term loss of responsiveness can be explained by death of the nociceptor or destruction of its peripheral terminals following exposure to capsaicin (Jancso, G. *et al.* (1977) *Nature* 270, 741-743).
- 25 The cellular specificity of capsaicin action and its ability to evoke the sensation of burning pain have led to speculation that the target of capsaicin action plays an important physiological role in the detection of painful stimuli. Indeed, capsaicin may elicit the perception of pain by mimicking the actions of a physiological stimulus or an endogenous ligand produced during tissue injury (James, I.F., Kinkina, N.N. & Wood, J.N. in *Capsaicin in the Study of Pain* (ed. Wood, J.N.) 83-104 (Academic, London,
30 1993).

Caterina M.J. *et al.* have recently determined the molecular basis underlying this phenomenon by characterizing a functional cDNA that encodes a vanilloid receptor (VR-1) in rat sensory ganglia (Caterina M. J. *et al.*, (1997) *Nature* 389:816-824). VR-1 is a vanilloid-gated, nonselective cation channel that resembles members of the transient receptor potential (TRP) channel family, first identified as components of the *Drosophila* phototransduction pathway (Montell *et al.* (1989) *Neuron* 2:1313-1323).

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Summary of the Invention

The present invention is based, at least in part, on the discovery of novel members of the Capsaicin/Vanilloid family of receptors. Described herein is the isolation of the human orthologue of rat VR-1 (rVR-1), referred to herein as hVR-1, as well as another previously unknown member of the VR family of receptors, referred herein as VR-2, and specifically as human VR-2 (hVR-2, including an alternate form which contains a deletion) and rat VR-2 (rVR-2) nucleic acid and protein molecules. The hVR-1, hVR-2, and rVR-2 molecules of the present invention are useful as targets for developing modulating agents to regulate a variety of cellular processes, *e.g.*, cellular processes involved in pain. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding hVR-1, hVR-2, and rVR-2 proteins and fragments thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of hVR-1, hVR-2, and rVR-2-encoding nucleic acids.

In one embodiment, an hVR-1, hVR-2, or rVR-2 nucleic acid molecule of the invention is at least 60%, 65%, 70%, 75%, 80%, 83%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 or a complement thereof.

In another embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a complement thereof. In another embodiment, the nucleic acid molecule includes at least 10, 15, 20, or more contiguous nucleotides of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

In another embodiment, an hVR-1, hVR-2, and rVR-2 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, 5, 8, or 11. In one embodiment, an hVR-1, hVR-2, and rVR-2 nucleic acid molecule includes a
5 nucleotide sequence encoding a protein having an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 95%, 98% or more identical to the entire length of the amino acid sequence of SEQ ID NO:2, 5, 8, or 11.

Another embodiment of the invention features nucleic acid molecules, preferably hVR-1, hVR-2, and rVR-2 nucleic acid molecules, which specifically detect hVR-1,
10 hVR-2, and rVR-2 nucleic acid molecules relative to nucleic acid molecules encoding non-hVR-1, non-hVR-2, and non-hVR-2 proteins. For example, in one embodiment, such a nucleic acid molecule is at least 100-150, 1150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-700, 700-800, 800-900, 900-1000, 1088, or more nucleotides in length and hybridizes under stringent conditions to a
15 nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-17, 3696-3863, or 3901-3909 of SEQ ID NO:1. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-17, 3696-3863, or
20 3901-3909 of SEQ ID NO:1. In yet other preferred embodiments, the nucleic acid molecules consist of nucleotides 1-17, 3696-3863, or 3901-3909 of SEQ ID NO:1. In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1944-2003 of SEQ ID NO:4. In other preferred embodiments, the nucleic acid molecules comprise
25 nucleotides 1944-2003 of SEQ ID NO:4. In yet other preferred embodiments, the nucleic acid molecules consist of nucleotides 1944-2003 of SEQ ID NO:4.

In other embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule
30 consisting of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 under stringent conditions and is encoded by the same locus as hVR-1, hVR-2 or rVR-2.

Another embodiment of the invention provides a nucleic acid molecule that encodes a naturally occurring orthologue of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule consisting of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 under stringent
5 conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to an hVR-1, hVR-2, and rVR-2 nucleic acid molecule, *e.g.*, the coding strand of an hVR-1, hVR-2, and rVR-2 nucleic acid molecule.

Since the hVR2 (the alternate form) and rVR2 sequences represent fragments of
10 the entire coding regions of these genes, another embodiment of the invention provides the complete gene sequences. A skilled artisan can readily isolate such molecule using the sequences disclosed herein.

Another aspect of the invention provides a vector comprising an hVR-1, an hVR-2, or a rVR-2 nucleic acid molecule. In certain embodiments, the vector is a
15 recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. In yet another embodiment, the invention provides a host cell containing a nucleic acid molecule of the invention. The invention also provides a method for producing a protein, preferably an hVR-1, hVR-2, and rVR-2 protein, by culturing in a suitable medium, a host cell, *e.g.*, a mammalian host cell such
20 as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the protein is produced.

Another aspect of this invention features isolated or recombinant hVR-1, hVR-2, and rVR-2 proteins and polypeptides. In one embodiment, the isolated protein, preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane
25 domain. In another embodiment, the isolated protein, preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane domain and at least one proline rich domain. In yet another embodiment, the isolated protein, preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane domain, at least one proline rich domain, and at least one ankyrin repeat domain. In yet another embodiment, the protein,
30 preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane domain, at least one proline rich domain, and at least one ankyrin repeat domain and has

an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 95%, 98% or more homologous to the amino acid sequence of SEQ ID NO:2, 5, 8, or 11. In another embodiment, the protein, preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane domain, at least one proline rich domain, and at least one ankyrin repeat domain and plays a role in the development and regulation of pain. In yet another embodiment, the protein, preferably an hVR-1, hVR-2, and rVR-2 protein, includes at least one transmembrane domain, at least one proline rich domain, and at least one ankyrin repeat domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

In another embodiment, the invention features fragments of the protein having the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the fragment comprises at least 15, 30, 40, 50, 60, 70, 80, 90, or 100 amino acids (*e.g.*, contiguous amino acids).

In another embodiment, the invention features an isolated protein, preferably an hVR-1, hVR-2, and rVR-2 protein, which is encoded by a nucleic acid molecule consisting of a nucleotide sequence at least about 60%, 65%, 70%, 75%, 80%, 83%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a complement thereof. This invention further features an isolated protein, preferably an hVR-1, hVR-2, or rVR-2 protein, which is encoded by a nucleic acid molecule consisting of a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule consisting of the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a complement thereof.

The proteins of the present invention or portions thereof, *e.g.*, biologically active portions thereof, can be operatively linked to a non-hVR-1, non-hVR-2, or non-rVR-2 polypeptide (*e.g.*, heterologous amino acid sequences) to form fusion proteins. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention, preferably hVR-1, hVR-2, and rVR-2 proteins. In addition, the hVR-1, hVR-2, and rVR-2 proteins or biologically active

portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of an hVR-1, hVR-2, and rVR-2 nucleic acid molecule, protein or polypeptide
5 in a biological sample by contacting the biological sample with an agent capable of detecting an hVR-1, hVR-2, and rVR-2 nucleic acid molecule, protein or polypeptide such that the presence of an hVR-1, hVR-2, and rVR-2 nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the
10 presence of hVR-1, hVR-2, and rVR-2 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of hVR-1, hVR-2, and rVR-2 activity such that the presence of hVR-1, hVR-2, and rVR-2 activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating hVR-1, hVR-
15 2, and rVR-2 activity comprising contacting a cell capable of expressing hVR-1, hVR-2, and rVR-2 with an agent that modulates hVR-1, hVR-2, and rVR-2 activity such that hVR-1, hVR-2, and rVR-2 activity in the cell is modulated. In one embodiment, the agent inhibits hVR-1, hVR-2, and rVR-2 activity. In another embodiment, the agent stimulates hVR-1, hVR-2, and rVR-2 activity. In one embodiment, the agent is an
20 antibody that specifically binds to an hVR-1, hVR-2, and rVR-2 protein. In another embodiment, the agent modulates expression of hVR-1, hVR-2, and rVR-2 by modulating transcription of an hVR-1, hVR-2, and rVR-2 gene or translation of an hVR-1, hVR-2, and rVR-2 mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of an hVR-
25 1, hVR-2, and rVR-2 mRNA or an hVR-1, hVR-2, and rVR-2 gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant hVR-1, hVR-2, and rVR-2 protein or nucleic acid expression or activity by administering an agent which is an hVR-1, hVR-2, and rVR-2 modulator to the subject. In one embodiment, the hVR-1, hVR-2, and rVR-2
30 modulator is an hVR-1, hVR-2, and rVR-2 protein. In another embodiment the hVR-1, hVR-2, and rVR-2 modulator is an hVR-1, hVR-2, and rVR-2 nucleic acid molecule. In

yet another embodiment, the hVR-1, hVR-2, and rVR-2 modulator is a peptide, peptidomimetic, or other small molecule. In a further embodiment, the disorder characterized by aberrant hVR-1, hVR-2, and rVR-2 protein or nucleic acid expression is a pain disorder, *e.g.*, hyperalgesia.

5 The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding an hVR-1, hVR-2, and rVR-2 protein; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of an hVR-1, hVR-2, and rVR-2 protein, wherein a wild-type form of the gene encodes a protein
10 with an hVR-1, hVR-2, and rVR-2 activity (as described herein).

 In another aspect the invention provides a method for identifying a compound that binds to or modulates the activity of an hVR-1, hVR-2, and rVR-2 protein, by providing an indicator composition comprising an hVR-1, hVR-2, and rVR-2 protein having hVR-1, hVR-2, and rVR-2 activity, contacting the indicator composition with a
15 test compound, and determining the effect of the test compound on hVR-1, hVR-2, and rVR-2 activity in the indicator composition to identify a compound that modulates the activity of an hVR-1, hVR-2, and rVR-2 protein.

 Other features and advantages of the invention will be apparent from the following detailed description and claims.

20

Brief Description of the Drawings

Figure 1 depicts the full length cDNA sequence and predicted amino acid sequence of human VR-1 (hVR-1). The nucleotide sequence corresponds to nucleic acids 1 to 3909 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids
25 1 to 839 of SEQ ID NO:2. The coding region without the 5' and 3' untranslated regions of the human VR-1 (hVR-1) gene is shown in SEQ ID NO:3.

Figure 2 depicts the full length cDNA sequence and predicted amino acid sequence of human VR-2 (hVR-2). The nucleotide sequence corresponds to nucleic acids 1 to 2809 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids
30 1 to 764 of SEQ ID NO:5. The coding region without the 5' and 3' untranslated regions of the human VR-2 (hVR-2) gene is shown in SEQ ID NO:6.

Figure 3 depicts the partial cDNA sequence and partial predicted amino acid sequence of an alternate form of human VR-2 (hVR-2). The nucleotide sequence corresponds to nucleic acids 1 to 1489 of SEQ ID NO:7. The amino acid sequence corresponds to amino acids 1 to 436 of SEQ ID NO:8. The coding region without the 5' and 3' untranslated regions of the alternate form of human VR-2 (hVR-2) gene is shown in SEQ ID NO:9.

Figure 4 depicts the partial cDNA sequence and partial predicted amino acid sequence of rat VR-2 (rVR-2). The nucleotide sequence corresponds to nucleic acids 1 to 1794 of SEQ ID NO:10. The amino acid sequence corresponds to amino acids 1 to 554 of SEQ ID NO:11. The coding region without the 5' and 3' untranslated regions of the rat VR-2 (rVR-2) gene is shown in SEQ ID NO:12.

Figure 5 depicts an alignment of the hVR-1 protein (SEQ ID NO:2) with the human VR-2 protein (SEQ ID NO:5) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4.

Figure 6 depicts an alignment of the hVR-1 nucleotide sequence (SEQ ID NO:1) with the human VR-2 nucleotide sequence (SEQ ID NO:4) using the GAP program in the GCG software package (nwsgapdna matrix) and a gap weight of 50 and a length weight of 3.

Figure 7 depicts an alignment of the hVR-2 protein (SEQ ID NO:5) with the rat VR-2 protein (SEQ ID NO:11) using the CLUSTAL W (1.74) multiple sequence alignment program.

Figure 8 depicts an alignment of the hVR-2 protein (SEQ ID NO:5) with the rat VR-2 protein (SEQ ID NO:11) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4.

Figure 9 depicts an alignment of the hVR-1 nucleotide sequence (SEQ ID NO:1) with the rat VR-1 nucleotide sequence (Accession Number:AF029310) using the GAP program in the GCG software package (nwsgapdna matrix) and a gap weight of 50 and a length weight of 3.

Figure 10 depicts an alignment of the hVR-1 protein (SEQ ID NO:2) with the rat VR-1 protein (Accession Number:AF029310) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4.

Figure 11 depicts an alignment of the hVR-2 protein (SEQ ID NO:5) with the human VR-2 protein (alternate form) (SEQ ID NO:8) using the CLUSTAL W (1.74) multiple sequence alignment program.

Figure 12 depicts a structural, hydrophobicity, and antigenicity analysis of the hVR-1 protein.

Figure 13 depicts the results of a search using the amino acid sequence of the hVR-1 protein against the HMM database.

Figure 14 depicts a structural, hydrophobicity, and antigenicity analysis of the hVR-2 protein.

Figure 15 depicts the results of a search using the amino acid sequence of the hVR-2 protein against the HMM database.

Figure 16 depicts the predicted full length amino acid sequence of the human VR-2 protein (alternate form) (SEQ ID NO:20).

Figure 17 depicts an alignment of the hVR-2 protein (SEQ ID NO:5) with the predicted full length human VR-2 protein (alternate form) (SEQ ID NO:20) using the CLUSTAL W (1.74) multiple sequence alignment program.

Detailed Description of the Invention

The present invention is based, at least in part, on the discovery of nucleic acid and amino acid molecules which are novel members of the Capsaicin/Vanilloid family of receptors. Described herein is the isolation of the human orthologue of rat VR-1 (rVR-1), referred to herein as hVR-1, as well as another previously unknown member of the VR family of receptors, referred to herein as VR-2, and specifically as human VR-2 (hVR-2) and rat VR-2 (rVR-2) nucleic acid and protein molecules. The hVR-1, hVR-2, and rVR-2 molecules were identified based on their sequence similarity to the known rat vanilloid receptor (VR-1). VR-1 is a vanilloid gated, non-selective cation channel which resembles members of the transient receptor potential (TRP) ion channel family (described in Montell *et al.* (1989) *Neuron* 2:1313-1323) that mediate the influx of extracellular calcium in response to depletion of intracellular calcium stores. The rat VR-1 cDNA contains an open reading frame of 2514 nucleotides that encodes a protein of 838 amino acids. Hydrophilicity analysis has indicated that rat VR-1 contains six

transmembrane domains (predicted to be mostly α -helices) with an additional short hydrophobic stretch between transmembrane regions 5 and 6. The amino terminal hydrophilic segment contains a relatively proline rich region followed by three ankyrin repeat domains. The rat VR-1 is expressed in small diameter neurons within sensory
5 ganglia. The present hVR-1 sequence is the human orthologue of rVR-1. As described in further detail *infra*, the human VR-1 is expressed in nodose, trigeminal sensory neurons, as well as in some, but not all, small dorsal root ganglion (DRG) neurons and in a few medium sized DRG neurons.

The hVR-1, hVR-2, and rVR-2 molecules of the present invention play a role in
10 pain signaling mechanisms. As used herein, the term "pain signaling mechanisms" includes the cellular mechanisms involved in the development and regulation of pain, *e.g.*, pain elicited by noxious chemical, mechanical, or thermal stimuli, in a subject, *e.g.*, a mammal such as a human. In mammals, the initial detection of noxious chemical, mechanical, or thermal stimuli, a process referred to as "nociception", occurs
15 predominantly at the peripheral terminals of specialized, small diameter primary afferent neurons, called polymodal nociceptors. These afferent neurons transmit the information to the central nervous system, evoking a perception of pain or discomfort and initiating appropriate protective reflexes. Capsaicin/Vanilloid receptors, *e.g.*, the hVR-1, hVR-2, and rVR-2 molecules of the present invention, present on these afferent neurons, are
20 involved in detecting these noxious chemical, mechanical, or thermal stimuli and transducing this information into membrane depolarization events. Thus, the hVR-1, hVR-2, and rVR-2 molecules by participating in pain signaling mechanisms, can modulate pain elicitation and provide novel diagnostic targets and therapeutic agents to control pain.

25 The hVR-1, hVR-2, and rVR-2 molecules provide novel diagnostic targets and therapeutic agents to control pain in a variety of disorders, diseases, or conditions which are characterized by a deregulated, *e.g.*, upregulated or downregulated, pain response. For example, the hVR-1, hVR-2, and rVR-2 molecules provide novel diagnostic targets and therapeutic agents to control the exaggerated pain response elicited during various
30 forms of tissue injury, *e.g.*, inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L. (1987) *Pain*, New York:McGraw-

Hill). Moreover, the hVR-1, hVR-2, and rVR-2 molecules provide novel diagnostic targets and therapeutic agents to control pain associated with musculoskeletal disorders, *e.g.*, joint pain; tooth pain; headaches; pain associated with surgery, or neuropathic pain.

As the hVR-1 gene maps to a region of human chromosome 17 between WI-
5 5436 (7.7cR) and WI-6584 (18.9cR) (Example 6), which has been associated with myasthenia gravis, Smith-Magenis syndrome, CORD5, Cone-rod dysrtrophy, and breast cancer, the hVR-1 molecule may provide novel diagnostic targets and therapeutic agents to treat, diagnose, or prognose these disorders or other disorders linked to this chromosomal region. Similarly, as the hVR-2 gene maps to a region of human
10 chromosome 17 between AFMA043ZB5 (23.3 cR) and D17S721 (29.3cR) (Example 6) which has been associated with myasthenia gravis, Smith-Magenis syndrome, CORD5, Cone-rod dysrtrophy, choroidal dystrophy, central areolar, and retinal cone dystrophy, the hVR-2 molecule may provide novel diagnostic targets and therapeutic agents to treat, diagnose, or prognose these disorders or other disorders linked to this chromosomal
15 region.

The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-
20 naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics.

For example, the family of hVR-1, hVR-2, and rVR-2 proteins comprise at least
25 one, and preferably six "transmembrane domains." As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 20, 25, 30, 35, 40, or 45 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in
30 hydrophobic residues, and typically have a helical structure. In a embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acid residues of a

transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, Zagotta W.N. et al, (1996) *Annual Rev. Neurosci.* 19: 235-63, the contents of which are incorporated herein by reference. Amino acid residues 434-455, 480-495, (509-531; based on
5 homology to the rat VR-1) or 514-531, (543-569; based on homology to the rat VR-1) or 538-555, (577-596; based on homology to the rat VR-1) or 580-599, and (656-683; based on homology to the rat VR-1) or 658-682 of hVR-1 (SEQ ID NO:2) and amino acid residues 391-410, 431-448, 459-476, 486-508, 538-556, and 621-645 of hVR-2 (SEQ ID NO:5) comprise transmembrane domains.

10 In another embodiment, an hVR-1, hVR-2, and rVR-2 of the present invention is identified based on the presence of a "proline rich domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "proline rich domain" includes an amino acid sequence of about 4-6 amino acid residues in length having the general sequence X-Pro-X-X-Pro-X (where X can be any amino acid). Proline rich
15 domains are usually located in a helical structure and bind through hydrophobic interactions to SH3 domains. SH3 domains recognize proline rich domains in both forward and reverse orientations. Proline rich domains are described in, for example, Sattler M. *et al.* (1998) *Leukemia* 12:637-644, the contents of which are incorporated herein by reference.

20 In another embodiment, an hVR-1, hVR-2, and rVR-2 of the present invention is identified based on the presence of an "ankyrin repeat domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "ankyrin repeat domain" includes a protein domain having an amino acid sequence of about 30-50 amino acid residues and having a bit score for the alignment of the sequence to the ankyrin repeat
25 domain (HMM) of at least 6. Preferably, an ankyrin repeat domain includes at least about 30-45, more preferably about 30-40 amino acid residues, or about 30-35 amino acids and has a bit score for the alignment of the sequence to the ankyrin repeat domain (HMM) of at least 3-10, more preferably 10-30, more preferably 30-50, even more preferably 50-75, 75-100, 100-200 or greater. The ankyrin repeat domain HMM has
30 been assigned the PFAM Accession PF00023 (<http://genome.wustl.edu/Pfam/.html>). Ankyrin repeats are involved in protein-protein interactions and are described in, for

example, Ketchum K.A *et al.* (1996) *FEBS Letters* 378:19-26, the contents of which are incorporated herein by reference.

To identify the presence of an ankyrin repeat domain in an hVR-1, hVR-2, and rVR-2 protein and make the determination that a protein of interest has a particular
5 profile, the amino acid sequence of the protein is searched against a database of HMMs (*e.g.*, the Pfam database, release 2.1) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.* (1990) *Meth.*
10 *Enzymol.* 183:146-159; Gribskov *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.* (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.* (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference. A search was performed against the HMM database resulting in the identification of three ankyrin repeat domains in the amino acid sequence of SEQ ID NO:2 (at about residues 201-233,
15 248-283, and 333-361) and SEQ ID NO:5 (at about residues 162-194, 208-243, and 293-328). The results of the searches are set forth in Figures 13 and 15.

Isolated proteins of the present invention, preferably hVR-1, hVR-2, and rVR-2 proteins, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2, 5, 8, or 11 or are encoded by a nucleotide sequence sufficiently identical to
20 SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural
25 domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% identity, preferably 60% identity, more preferably 70%-80%, and even more preferably 90-95% identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently
30 identical. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%,

or 50%, preferably 60%, more preferably 70-80%, or 90-95% identity and share a common functional activity are defined herein as sufficiently identical.

As used interchangeably herein, an "hVR-1, hVR-2, and rVR-2 activity", "biological activity of hVR-1, hVR-2, and rVR-2" or "functional activity of hVR-1, hVR-2, and rVR-2",
5 refers to an activity exerted by an hVR-1, hVR-2, and rVR-2 protein, polypeptide or nucleic acid molecule on an hVR-1, hVR-2, and rVR-2 responsive cell or on an hVR-1, hVR-2, and rVR-2 protein substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, an hVR-1, hVR-2, and rVR-2 activity is a direct activity, such as an association with an hVR-1, hVR-2, and rVR-2-target molecule. As used herein, a "target
10 molecule" or "binding partner" is a molecule with which an hVR-1, hVR-2, and rVR-2 protein binds or interacts in nature, such that hVR-1, hVR-2, and rVR-2-mediated function is achieved. An hVR-1, hVR-2, and rVR-2 target molecule can be a non-hVR-1, non-hVR-2, and non-rVR-2 molecule or an hVR-1, hVR-2, and rVR-2 protein or polypeptide of the present invention. In an exemplary embodiment, an hVR-1, hVR-2, and rVR-2 target
15 molecule is an hVR-1, hVR-2, and rVR-2 ligand, *e.g.*, capsaicin. Alternatively, an hVR-1, hVR-2, and rVR-2 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the hVR-1, hVR-2, and rVR-2 protein with an hVR-1, hVR-2, and rVR-2 ligand.

Accordingly, another embodiment of the invention features isolated hVR-1,
20 hVR-2, and rVR-2 proteins and polypeptides having an hVR-1, hVR-2, and rVR-2 activity. Other proteins of the invention are hVR-1, hVR-2, and rVR-2 proteins having at least one, and preferably six, transmembrane domains and, preferably, an hVR-1, hVR-2, and rVR-2 activity. Yet other proteins of the invention are hVR-1, hVR-2, and rVR-2 proteins having at least one transmembrane domain, at least one proline rich
25 domain and, preferably, an hVR-1, hVR-2, and rVR-2 activity. Other proteins of the invention are hVR-1, hVR-2, and rVR-2 proteins having at least one transmembrane domain, at least one proline rich domain, at least one ankyrin repeat domain and, preferably, an hVR-1, hVR-2, and rVR-2 activity. Additional proteins of the invention have at least one transmembrane domain, at least one proline rich domain, at least one
30 ankyrin repeat domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a

nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

The nucleotide sequence of the full length hVR-1 cDNA and the predicted amino acid sequence of the hVR-1 polypeptide are shown in Figure 1 and in SEQ ID
5 NOs:1 and 2, respectively.

The nucleotide sequence of the full length hVR-2 cDNA and the predicted amino acid sequence of the hVR-2 polypeptide are shown in Figure 2 and in SEQ ID NOs:4 and 5, respectively.

The nucleotide sequence of the partial hVR-2 (alternate form) cDNA and the
10 predicted amino acid sequence of the hVR-2 (alternate form) polypeptide are shown in Figure 3 and in SEQ ID NOs:7 and 8, respectively.

The nucleotide sequence of the partial rVR-2 cDNA and the predicted amino acid sequence of the rVR-2 polypeptide are shown in Figure 4 and in SEQ ID NOs:10 and 11, respectively.

15 Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that
20 encode hVR-1, hVR-2, and rVR-2 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify hVR-1, hVR-2, and rVR-2-encoding nucleic acid molecules (*e.g.*, hVR-1, hVR-2, and rVR-2 mRNA) and fragments for use as PCR primers for the amplification or mutation of hVR-1, hVR-2, and rVR-2 nucleic acid molecules. As used herein, the term "nucleic acid
25 molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which
30 are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated"

includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated hVR-1, hVR-2, and rVR-2 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, as a hybridization probe, hVR-1, hVR-2, and rVR-2 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to hVR-1, hVR-2, and rVR-2 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In one embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the full length hVR-1 encoding cDNA.

5 In another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:4. The sequence of SEQ ID NO:4 corresponds to the full length hVR-2 encoding cDNA.

In another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:7. The sequence of SEQ ID NO:7 corresponds to a fragment of the hVR-2 (alternate form) encoding cDNA.

10 In another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:10. The sequence of SEQ ID NO:10 corresponds to a fragment of the rVR-2 cDNA.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 thereby forming a stable duplex.

20 In still another embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 60%, 65%, 70%, 75%, 80%, 83%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to the entire length of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of an hVR-1, hVR-2, and rVR-2 protein, *e.g.*, a biologically active portion of an hVR-1, hVR-2, and rVR-2 protein. The nucleotide sequence determined from the cloning of the hVR-1, hVR-2, and rVR-2 gene allows for the generation of probes and primers

designed for use in identifying and/or cloning other hVR-1, hVR-2, and rVR-2 family members, as well as hVR-1, hVR-2, and rVR-2 homologues from other species. The probe/primer typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes
5 under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, 75, or 100 consecutive nucleotides of a sense sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, of an anti-sense sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. In an exemplary embodiment, a nucleic acid
10 molecule of the present invention comprises a nucleotide sequence which is greater than 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1088, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

15 Probes based on the hVR-1, hVR-2, and rVR-2 nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for
20 identifying cells or tissue which misexpress an hVR-1, hVR-2, and rVR-2 protein, such as by measuring a level of an hVR-1, hVR-2, and rVR-2-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting hVR-1, hVR-2, and rVR-2 mRNA levels or determining whether a genomic hVR-1, hVR-2, and rVR-2 gene has been mutated or deleted.

25 A nucleic acid fragment encoding a "biologically active portion of an hVR-1, hVR-2, and rVR-2 protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, which encodes a polypeptide having an hVR-1, hVR-2, and rVR-2 biological activity (the biological activities of the hVR-1, hVR-2, and rVR-2 proteins are described herein), expressing the encoded portion of the
30 hVR-1, hVR-2, and rVR-2 protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the hVR-1, hVR-2, and rVR-2 protein.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, due to degeneracy of the genetic code and thus encode the same hVR-1, hVR-2, and rVR-2 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, 5, 8, or 11.

In addition to the hVR-1, hVR-2, and rVR-2 nucleotide sequences shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the hVR-1, hVR-2, and rVR-2 proteins may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the hVR-1, hVR-2, and rVR-2 genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding an hVR-1, hVR-2, and rVR-2 protein, preferably a mammalian hVR-1, hVR-2, and rVR-2 protein, and can further include non-coding regulatory sequences, and introns.

Allelic variants of hVR-1, hVR-2, and rVR-2 include both functional and non-functional hVR-1, hVR-2, and rVR-2 proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the hVR-1, hVR-2, and rVR-2 protein that maintain the ability to bind an hVR-1, hVR-2, and rVR-2 ligand and/or modulate a pain signaling mechanism. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, 5, 8, or 11, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the hVR-1, hVR-2, and rVR-2 protein that do not have the ability to either bind an hVR-1, hVR-2, and rVR-2 ligand and/or modulate a pain signaling mechanism. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID

NO:2, 5, 8, or 11, or a substitution, insertion or deletion in critical residues or critical regions.

The present invention further provides non-human orthologues of the hVR-2 and rVR-2 protein. Orthologues of the hVR-2 and rVR-2 protein are proteins that are
5 isolated from non-human and non-rat organisms and possess the same hVR-2 and rVR-2 ligand binding and/or modulation of pain signaling mechanism capabilities of the hVR-2 and rVR-2 proteins. Orthologues of the hVR-2 and rVR-2 proteins can readily be identified as comprising an amino acid sequence that is substantially homologous to SEQ ID NO: 4, 6, 8 or 10.

10 Moreover, nucleic acid molecules encoding other hVR-1, hVR-2, and rVR-2 family members and, thus, which have a nucleotide sequence which differs from the hVR-1, hVR-2, and rVR-2 sequences of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, are intended to be within the scope of the invention. For example, another hVR-1, hVR-2, and rVR-2 cDNA can be identified based on the nucleotide sequence of hVR-1, hVR-2,
15 and rVR-2. Moreover, nucleic acid molecules encoding VR-2 proteins from different species, and which, thus, have a nucleotide sequence which differs from the hVR-2 and rVR-2 sequences of SEQ ID NO:4, 6, 8, or 10 are intended to be within the scope of the invention. For example, a mouse hVR-2 cDNA can be identified based on the nucleotide sequence of the human VR-2 (hVR-2) or the rat VR-2 (rVR-2).

20 Nucleic acid molecules corresponding to natural allelic variants and homologues of the hVR-1, hVR-2, and rVR-2 cDNAs of the invention can be isolated based on their homology to the hVR-1, hVR-2, and rVR-2 nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid
25 molecules corresponding to natural allelic variants and homologues of the hVR-1, hVR-2, and rVR-2 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the hVR-1, hVR-2, and rVR-2 gene.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under
30 stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. In other embodiment, the nucleic acid is at least

30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or 950 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% identical to each other typically remain

5 hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-

10 limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50°C, preferably at 55°C, more preferably at 60°C, and even more preferably at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10,

15 or 12 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In addition to naturally-occurring allelic variants of the hVR-1, hVR-2, and rVR-2 sequences that may exist in the population, the skilled artisan will further appreciate that

20 changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, thereby leading to changes in the amino acid sequence of the encoded hVR-1, hVR-2, and rVR-2 proteins, without altering the functional ability of the hVR-1, hVR-2, and rVR-2 proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ

25 ID NO:1, 3, 4, 6, 7, 9, 10, or 12. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of hVR-1, hVR-2, and rVR-2 (*e.g.*, the sequence of SEQ ID NO:2, 5, 8, or 11) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the hVR-1, hVR-2, and rVR-2 proteins of the

30 present invention, are predicted to be particularly unamenable to alteration. Furthermore,

additional amino acid residues that are conserved between the hVR-1, hVR-2, and rVR-2 proteins of the present invention and other members of the Capsaicin/Vanilloid receptor family are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules
5 encoding hVR-1, hVR-2, and rVR-2 proteins that contain changes in amino acid residues that are not essential for activity. Such hVR-1, hVR-2, and rVR-2 proteins differ in amino acid sequence from SEQ ID NO:2, 5, 8, or 11, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about
10 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 95%, 98% or more homologous to SEQ ID NO:2, 5, 8, or 11.

An isolated nucleic acid molecule encoding an hVR-1, hVR-2, and rVR-2 protein homologous to the protein of SEQ ID NO:2, 5, 8, or 11 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of
15 SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid
20 residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*,
25 glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an hVR-1, hVR-2, and rVR-2 protein is preferably replaced with
30 another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an hVR-1, hVR-

2, and rVR-2 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for hVR-1, hVR-2, and rVR-2 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

5 In an embodiment, a mutant hVR-1, hVR-2, and rVR-2 protein can be assayed for the ability to (1) interact with a non-hVR-1, non-hVR-2, or non-rVR-2 protein molecule, *e.g.*, a vanilloid compound such as capsaicin; (2) modulate intracellular calcium concentration; (3) activate an hVR-1, hVR-2, and rVR-2-dependent signal transduction pathway; or (4) modulate a pain signaling mechanism.

10 In addition to the nucleic acid molecules encoding hVR-1, hVR-2, and rVR-2 proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or
15 complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire hVR-1, hVR-2, and rVR-2 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding hVR-1, hVR-2, and rVR-2. The term
20 "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of hVR-1, hVR-2, and rVR-2). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding hVR-1, hVR-2, and rVR-2. The term "noncoding region" refers to 5' and 3' sequences which flank the
25 coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding hVR-1, hVR-2, and rVR-2 disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be
30 complementary to the entire coding region of hVR-1, hVR-2, and rVR-2 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding

or noncoding region of hVR-1, hVR-2, and rVR-2 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of hVR-1, hVR-2, and rVR-2 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (*v*), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (*v*), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)*w*, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an hVR-1, hVR-2, and rVR-2 protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The

hybridization can be by conventional nucleotide complementarity to form a stable duplex. or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes. through specific interactions in the major groove of the double helix.

An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-*o*-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave hVR-1, hVR-2, and rVR-2 mRNA transcripts to thereby inhibit translation of hVR-1, hVR-2, and rVR-2 mRNA. A ribozyme having specificity for an hVR-1, hVR-2, and rVR-2-encoding nucleic acid can be designed based upon the nucleotide sequence of an hVR-1, hVR-2, and rVR-2 cDNA disclosed herein (*i.e.*, SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12). For example, a derivative of a *Tetrahymena* L-19 IVS

RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an hVR-1, hVR-2, and rVR-2-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, hVR-1, hVR-2, and rVR-2 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, for example, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, hVR-1, hVR-2, and rVR-2 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the hVR-1, hVR-2, and rVR-2 (*e.g.*, the hVR-1, hVR-2, and rVR-2 promoter and/or enhancers) to form triple helical structures that prevent transcription of the hVR-1, hVR-2, and rVR-2 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In yet another embodiment, the hVR-1, hVR-2, and rVR-2 nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs of hVR-1, hVR-2, and rVR-2 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of hVR-1,

hVR-2, and rVR-2 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. 5 *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of hVR-1, hVR-2, and rVR-2 can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras 10 of hVR-1, hVR-2, and rVR-2 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms 15 of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4- 20 methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' 25 PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; 30 PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-

triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

5

II. Isolated hVR-1, hVR-2, and rVR-2 Proteins and Anti-hVR-1, Anti-hVR-2, and Anti-rVR-2 Antibodies

One aspect of the invention pertains to isolated hVR-1, hVR-2, and rVR-2 proteins, and biologically active portions thereof, as well as polypeptide fragments
10 suitable for use as immunogens to raise anti-hVR-2, anti-hVR-2, and anti-rVR-2 antibodies. In one embodiment, native hVR-1, hVR-2, and rVR-2 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, hVR-1, hVR-2, and rVR-2 proteins are produced by recombinant DNA techniques. Alternative to
15 recombinant expression, an hVR-1, hVR-2, and rVR-2 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the hVR-1, hVR-2, and rVR-2 protein is derived, or
20 substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of hVR-1, hVR-2, and rVR-2 protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations
25 of hVR-1, hVR-2, and rVR-2 protein having less than about 30% (by dry weight) of non-hVR-1, hVR-2, and rVR-2 protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-hVR-1, hVR-2, and rVR-2 protein, still more preferably less than about 10% of non-hVR-1, hVR-2, and rVR-2 protein, and most preferably less than about 5% non-hVR-1, non-hVR-2, and non-rVR-2
30 protein. When the hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture

medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of hVR-1, hVR-2, and rVR-2 protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of hVR-1, hVR-2, and rVR-2 protein having less than about 30% (by dry weight) of chemical precursors or non-hVR-1, hVR-2, and rVR-2 chemicals, more preferably less than about 20% chemical precursors or non-hVR-1, hVR-2, and rVR-2 chemicals, still more preferably less than about 10% chemical precursors or non-hVR-1, hVR-2, and rVR-2 chemicals, and most preferably less than about 5% chemical precursors or non-hVR-1, hVR-2, and rVR-2 chemicals.

As used herein, a "biologically active portion" of an hVR-1, hVR-2, and rVR-2 protein includes a fragment of an hVR-1, hVR-2, and rVR-2 protein which participates in an interaction between an hVR-1, hVR-2, and rVR-2 molecule and a non-hVR-1, non-hVR-2, and non-rVR-2 molecule, respectively. Biologically active portions of an hVR-1, hVR-2, and rVR-2 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the hVR-1, hVR-2, and rVR-2 protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, 5, 8, or 11, which include less amino acids than the full length hVR-1, hVR-2, and rVR-2 proteins, and exhibit at least one activity of an hVR-1, hVR-2, and rVR-2 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the hVR-1, hVR-2, and rVR-2 protein, *e.g.*, binding of an hVR-1, hVR-2, and rVR-2 ligand such as a vanilloid compound, *e.g.*, Capsaicin. A biologically active portion of an hVR-1, hVR-2, and rVR-2 protein can be a polypeptide which is, for example, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200 or more amino acids in length. Biologically active portions of an hVR-1, hVR-2, and rVR-2 protein can be used as targets for developing agents which modulate an hVR-1, hVR-2, and rVR-2 mediated activity, *e.g.*, a pain signaling mechanism.

In one embodiment, a biologically active portion of an hVR-1, hVR-2, and rVR-2 protein comprises at least one transmembrane domain, and/or at least one proline rich domain, and/or at least one ankyrin repeat domain. It is to be understood that a biologically active portion of an hVR-1, hVR-2, and rVR-2 protein of the present invention may contain at least one of the above-identified structural domains. A more biologically active portion of an hVR-1, hVR-2, and rVR-2 protein may contain at least two of the above-identified structural domains. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native hVR-1, hVR-2, and rVR-2 protein.

In a embodiment, the hVR-1, hVR-2, and rVR-2 protein has an amino acid sequence shown in SEQ ID NO:2, 5, 8, or 11. In other embodiments, the hVR-1, hVR-2, and rVR-2 protein is substantially homologous to SEQ ID NO:2, 5, 8, or 11, and retains the functional activity of the protein of SEQ ID NO:2, 5, 8, or 11, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the hVR-1, hVR-2, and rVR-2 protein is a protein which comprises an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 95%, 98% or more homologous to SEQ ID NO:2, 5, 8, or 11.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the hVR-1, hVR-2, and rVR-2 amino acid sequence of SEQ ID NO:2, 5, 8, or 11, having 177 amino acid residues, at least 80, preferably at least 100, more preferably at least 120, even more preferably at least 140, and even more preferably at least 150, 160 or 170 amino acid residues are aligned). The amino acid residues or nucleotides at

corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to hVR-1, hVR-2, and rVR-2 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50,

wordlength = 3 to obtain amino acid sequences homologous to hVR-1, hVR-2, and rVR-2 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

The invention also provides hVR-1, hVR-2, and rVR-2 chimeric or fusion proteins. As used herein, an hVR-1, hVR-2, and rVR-2 "chimeric protein" or "fusion protein" comprises an hVR-1, hVR-2, and rVR-2 polypeptide operatively linked to a non-hVR-1, hVR-2, and rVR-2 polypeptide. An "hVR-1, hVR-2, and rVR-2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to hVR-1, hVR-2, and rVR-2, whereas a "non-hVR-1, non-hVR-2, and non-rVR-2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the hVR-1, hVR-2, and rVR-2 protein, *e.g.*, a protein which is different from the hVR-1, hVR-2, and rVR-2 protein and which is derived from the same or a different organism. Within an hVR-1, hVR-2, and rVR-2 fusion protein the hVR-1, hVR-2, and rVR-2 polypeptide can correspond to all or a portion of an hVR-1, hVR-2, and rVR-2 protein. In a embodiment, an hVR-1, hVR-2, and rVR-2 fusion protein comprises at least one biologically active portion of an hVR-1, hVR-2, and rVR-2 protein. In another embodiment, an hVR-1, hVR-2, and rVR-2 fusion protein comprises at least two biologically active portions of an hVR-1, hVR-2, and rVR-2 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the hVR-1, hVR-2, and rVR-2 polypeptide and the non-hVR-1, non-hVR-2, and non-rVR-2 polypeptide are fused in-frame to each other. The non-hVR-1, hVR-2, and rVR-2 polypeptide can be fused to the N-terminus or C-terminus of the hVR-1, hVR-2, and rVR-2 polypeptide.

For example, in one embodiment, the fusion protein is a GST-hVR-1, GST-hVR-2, and GST-rVR-2 fusion protein in which the hVR-1, hVR-2, and rVR-2 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant hVR-1, hVR-2, and rVR-2.

In another embodiment, the fusion protein is an hVR-1, hVR-2, and rVR-2 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of hVR-1, hVR-2, and rVR-2 can be increased through use of a heterologous signal sequence.

5 The hVR-1, hVR-2, and rVR-2 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The hVR-1, hVR-2, and rVR-2 fusion proteins can be used to affect the bioavailability of an hVR-1, hVR-2, and rVR-2 substrate. Use of hVR-1, hVR-2, and rVR-2 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for
10 example, (i) aberrant modification or mutation of a gene encoding an hVR-1, hVR-2, and rVR-2 protein; (ii) mis-regulation of the hVR-1, hVR-2, and rVR-2 gene; and (iii) aberrant post-translational modification of an hVR-1, hVR-2, and rVR-2 protein.

Moreover, the hVR-1, hVR-2, and rVR-2-fusion proteins of the invention can be used as immunogens to produce anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies in a
15 subject, to purify hVR-1, hVR-2, and rVR-2 ligands and in screening assays to identify molecules which inhibit the interaction of hVR-1, hVR-2, and rVR-2 with an hVR-1, hVR-2, and rVR-2 substrate.

Preferably, an hVR-1, hVR-2, and rVR-2 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA
20 fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment,
25 the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular*
30 *Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST

polypeptide). An hVR-1, hVR-2, and rVR-2-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the hVR-1, hVR-2, and rVR-2 protein.

The present invention also pertains to variants of the hVR-1, hVR-2, and rVR-2
5 proteins which function as either hVR-1, hVR-2, and rVR-2 agonists (mimetics) or as hVR-1, hVR-2, and rVR-2 antagonists. Variants of the hVR-1, hVR-2, and rVR-2 proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of an hVR-1, hVR-2, and rVR-2 protein. An agonist of the hVR-1, hVR-2, and rVR-2 proteins can retain substantially the same, or a subset, of the biological activities of the
10 naturally occurring form of an hVR-1, hVR-2, and rVR-2 protein. An antagonist of an hVR-1, hVR-2, and rVR-2 protein can inhibit one or more of the activities of the naturally occurring form of the hVR-1, hVR-2, and rVR-2 protein by, for example, competitively modulating an hVR-1, hVR-2, and rVR-2-mediated activity of an hVR-1, hVR-2, and rVR-2 protein. Thus, specific biological effects can be elicited by treatment
15 with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the hVR-1, hVR-2, and rVR-2 protein.

In one embodiment, variants of an hVR-1, hVR-2, and rVR-2 protein which
20 function as either hVR-1, hVR-2, and rVR-2 agonists (mimetics) or as hVR-1, hVR-2, and rVR-2 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of an hVR-1, hVR-2, and rVR-2 protein for hVR-1, hVR-2, and rVR-2 protein agonist or antagonist activity. In one embodiment, a variegated library of hVR-1, hVR-2, and rVR-2 variants is generated by combinatorial mutagenesis at the
25 nucleic acid level and is encoded by a variegated gene library. A variegated library of hVR-1, hVR-2, and rVR-2 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential hVR-1, hVR-2, and rVR-2 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage
30 display) containing the set of hVR-1, hVR-2, and rVR-2 sequences therein. There are a variety of methods which can be used to produce libraries of potential hVR-1, hVR-2,

and rVR-2 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential hVR-1, hVR-2, and rVR-2 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of an hVR-1, hVR-2, and rVR-2 protein coding sequence can be used to generate a variegated population of hVR-1, hVR-2, and rVR-2 fragments for screening and subsequent selection of variants of an hVR-1, hVR-2, and rVR-2 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an hVR-1, hVR-2, and rVR-2 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the hVR-1, hVR-2, and rVR-2 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of hVR-1, hVR-2, and rVR-2 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis

(REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify hVR-1, hVR-2, and rVR-2 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

5 In one embodiment, cell based assays can be exploited to analyze a variegated hVR-1, hVR-2, and rVR-2 library. For example, a library of expression vectors can be transfected into a cell line, *e.g.*, a neuronal cell line, which ordinarily responds to a particular ligand in an hVR-1, hVR-2, and rVR-2-dependent manner. The transfected cells are then contacted with the ligand and the effect of expression of the mutant on
10 signaling by the ligand can be detected, *e.g.*, by measuring intracellular calcium concentration, neuronal membrane depolarization, or the activity of an hVR-1, hVR-2, and rVR-2-regulated transcription factor. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the ligand, and the individual clones further characterized.

15 An isolated hVR-1, hVR-2, and rVR-2 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind hVR-1, hVR-2, and rVR-2 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length hVR-1, hVR-2, and rVR-2 protein can be used or, alternatively, the invention provides antigenic peptide fragments of hVR-1, hVR-2, and rVR-2 for use as
20 immunogens. The antigenic peptide of hVR-1, hVR-2, and rVR-2 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, 5, 8, or 11 and encompasses an epitope of hVR-1, hVR-2, and rVR-2 such that an antibody raised against the peptide forms a specific immune complex with hVR-1, hVR-2, and rVR-2. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more
25 preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

 Epitopes encompassed by the antigenic peptide are regions of hVR-1, hVR-2, and rVR-2 that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity (see, for example, Figures 12 and 14).

An hVR-1, hVR-2, and rVR-2 immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed hVR-1, hVR-2, and rVR-2 protein or a chemically synthesized hVR-1, hVR-2, and rVR-2 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic hVR-1, hVR-2, and rVR-2 preparation induces a polyclonal anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody response.

Accordingly, another aspect of the invention pertains to anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as hVR-1, hVR-2, and rVR-2. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind hVR-1, hVR-2, and rVR-2. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of hVR-1, hVR-2, and rVR-2. A monoclonal antibody composition thus typically displays a single binding affinity for a particular hVR-1, hVR-2, and rVR-2 protein with which it immunoreacts.

Polyclonal anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies can be prepared as described above by immunizing a suitable subject with an hVR-1, hVR-2, and rVR-2 immunogen. The anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized hVR-1, hVR-2, and rVR-2. If desired, the antibody molecules directed against hVR-1, hVR-2, and rVR-2 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an

appropriate time after immunization, *e.g.*, when the anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 5 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing 10 monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:23136). Briefly, an immortal cell line 15 (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an hVR-1, hVR-2, and rVR-2 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds hVR-1, hVR-2, and rVR-2.

Any of the many well known protocols used for fusing lymphocytes and 20 immortalized cell lines can be applied for the purpose of generating an anti-hVR-1, anti-hVR-2, and anti-rVR-2 monoclonal antibodies (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also 25 would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium 30 containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard

techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind hVR-1, hVR-2, and rVR-2, *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with hVR-1, hVR-2, and rVR-2 to thereby isolate immunoglobulin library members that bind hVR-1, hVR-2, and rVR-2. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.*

(1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.

Additionally, recombinant anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and
5 non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M.,
10 European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc.*
15 *Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J.*
20 *Immunol.* 141:4053-4060.

An anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody (*e.g.*, monoclonal antibody) can be used to isolate hVR-1, hVR-2, and rVR-2 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody can facilitate the purification of natural hVR-1, hVR-2, and rVR-2
25 from cells and of recombinantly produced hVR-1, hVR-2, and rVR-2 expressed in host cells. Moreover, an anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody can be used to detect hVR-1, hVR-2, and rVR-2 protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the hVR-1, hVR-2, and rVR-2 protein. Anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies can be used
30 diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can

be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an hVR-1, hVR-2, and rVR-2 protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective

retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, hVR-1, hVR-2, and rVR-2 proteins, mutant forms of hVR-1, hVR-2, and rVR-2 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of hVR-1, hVR-2, and rVR-2 proteins in prokaryotic or eukaryotic cells. For example, hVR-1, hVR-2, and rVR-2 proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the

recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in hVR-1, hVR-2, and rVR-2 activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to, for example, generate antibodies specific for hVR-1, hVR-2, and rVR-2 proteins. In a embodiment, an hVR-1, hVR-2, and rVR-2 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid *trp-lac* fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 *gn10-lac* fusion promoter mediated by a coexpressed viral RNA polymerase (T7 *gn1*). This viral

polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 *gn1* gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express
5 the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those
10 preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the hVR-1, hVR-2, and rVR-2 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include
15 pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

Alternatively, hVR-1, hVR-2, and rVR-2 proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression
20 of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian
25 expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both
30 prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed.*, Cold Spring

Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The expression characteristics of an endogenous hVR-1, hVR-2, and rVR-2 gene within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous hVR-1, hVR-2, and rVR-2 gene. For example, an endogenous hVR-1, hVR-2, and rVR-2 gene which is normally "transcriptionally silent", *i.e.*, a hVR-1, hVR-2, and rVR-2 gene which is normally not expressed, or is expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a transcriptionally silent, endogenous hVR-1, hVR-2, and rVR-2 gene, may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous hVR-1, hVR-2, and rVR-2 gene, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described *e.g.*, in Chappel, U.S.

5 Patent No.: 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an

10 RNA molecule which is antisense to hVR-1, hVR-2, and rVR-2 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific

15 expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*,

20 Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which an hVR-1, hVR-2, and rVR-2 nucleic acid molecule of the invention is introduced, *e.g.*, an hVR-1, hVR-2, and rVR-2 nucleic acid molecule within a recombinant expression vector or an hVR-

25 1, hVR-2, and rVR-2 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding

30 generations due to either mutation or environmental influences, such progeny may not,

in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, an hVR-1, hVR-2, and rVR-2 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells).
5 Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including
10 calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor*
15 *Laboratory Press, Cold Spring Harbor, NY, 1989*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is
20 generally introduced into the host cells along with the gene of interest. selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding an hVR-1, hVR-2, and rVR-2 protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic
25 acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) an hVR-1, hVR-2, and rVR-2 protein. Accordingly, the invention further provides methods for producing an hVR-1, hVR-2,
30 and rVR-2 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression

vector encoding an hVR-1, hVR-2, and rVR-2 protein has been introduced) in a suitable medium such that an hVR-1, hVR-2, and rVR-2 protein is produced. In another embodiment, the method further comprises isolating an hVR-1, hVR-2, and rVR-2 protein from the medium or the host cell.

5 The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which hVR-1, hVR-2, and rVR-2-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous hVR-1, hVR-2, and rVR-2 sequences have been
10 introduced into their genome or homologous recombinant animals in which endogenous hVR-1, hVR-2, and rVR-2 sequences have been altered. Such animals are useful for studying the function and/or activity of an hVR-1, hVR-2, and rVR-2 and for identifying and/or evaluating modulators of hVR-1, hVR-2, and rVR-2 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a
15 rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression
20 of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous hVR-1, hVR-2, and rVR-2 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an
25 embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing an hVR-1, hVR-2, and rVR-2-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The hVR-1, hVR-2, and rVR-2 cDNA sequence
30 of SEQ ID NO:1, 3, 5, 7 or 9 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a hVR-2 gene, such as a

mouse or rat hVR-2, *e.g.*, the rVR-2 gene, can be used as a transgene. Alternatively, an hVR-1, hVR-2, and rVR-2 gene homologue, such as another member of the Capsaicin/Vanilloid family, can be isolated based on hybridization to the hVR-1, hVR-2, and rVR-2 cDNA sequences of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, (described further
5 in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to an hVR-1, hVR-2, and rVR-2 transgene to direct expression of an hVR-1, hVR-2, and rVR-2 protein to particular cells. Methods for generating transgenic animals via embryo
10 manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other
15 transgenic animals. A transgenic founder animal can be identified based upon the presence of an hVR-1, hVR-2, and rVR-2 transgene in its genome and/or expression of hVR-1, hVR-2, and rVR-2 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding an hVR-1, hVR-2, and
20 rVR-2 protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an hVR-1, hVR-2, and rVR-2 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the hVR-1, hVR-2, and rVR-2 gene. The VR-1 or VR-2 gene can be a human gene (*e.g.*, the cDNA
25 of SEQ ID NO:1, 3, 5, 4, 6, 7, or 9), but more preferably, is a non-human homologue of a hVR-1 and hVR-2 gene (*e.g.*, the cDNA of SEQ ID NO:10 or 12, or a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO: 1, 3, 5, 4, 6, 7, or 9). For example, a mouse VR-2 gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous
30 VR-2 gene in the mouse genome. In an embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the

endogenous hVR-1, hVR-2, and rVR-2 gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous hVR-1, hVR-2, and rVR-2 gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous hVR-1, hVR-2, and rVR-2 protein). In the homologous recombination nucleic acid molecule, the altered portion of the hVR-1, hVR-2, and rVR-2 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the hVR-1, hVR-2, and rVR-2 gene to allow for homologous recombination to occur between the exogenous hVR-1, hVR-2, and rVR-2 gene carried by the homologous recombination nucleic acid molecule and an endogenous hVR-1, hVR-2, and rVR-2 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking hVR-1, hVR-2, and rVR-2 nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced hVR-1, hVR-2, and rVR-2 gene has homologously recombined with the endogenous hVR-1, hVR-2, and rVR-2 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT

International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One
5 example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the
10 transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

15 Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through
20 the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

25

IV. Pharmaceutical Compositions

The hVR-1, hVR-2, and rVR-2 nucleic acid molecules, fragments of hVR-1, hVR-2, and rVR-2 proteins, and anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into
30 pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically

acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for

example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of
5 surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be
10 brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a fragment of an hVR-1, hVR-2, and rVR-2 protein or an anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody) in the required amount in an appropriate solvent
15 with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum
20 drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and
25 used in the form of tablets, troches, or capsules. oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the
30 following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or

lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

- 5 For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

 Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be
10 permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in
15 the art.

 The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

- In one embodiment, the active compounds are prepared with carriers that will
20 protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.
25 The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound

5 calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

10 Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio
15 LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in
20 formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially
25 from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high
30 performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 5 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can 10 include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated 15 that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small 20 molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per 25 mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents depends upon a 30 number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon

the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

5 Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the
10 potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein.

When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example,
15 prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration,
20 the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by
25 stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the
30 pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

5 The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, an hVR-1, hVR-2, and rVR-2 protein of the
10 invention has one or more of the following activities: (1) it interacts with a non-hVR-1, non-hVR-2, and non-rVR-2 protein molecule, *e.g.*, a vanilloid compound such as capsaicin; (2) it modulates intracellular calcium concentration; (3) it activates an hVR-1, hVR-2, and rVR-2-dependent signal transduction pathway; and (4) it modulates a pain signaling mechanism, and, thus, can be used to, for example, (1) modulate the
15 interaction with a non-hVR-1, non-hVR-2, and non-rVR-2 protein molecule; (2) modulate intracellular calcium concentration; (3) activate an hVR-1, hVR-2, and rVR-2-dependent signal transduction pathway; and (4) modulate a pain signaling mechanism.

 The isolated nucleic acid molecules of the invention can be used, for example, to express hVR-1, hVR-2, and rVR-2 protein (*e.g.*, via a recombinant expression vector in
20 a host cell in gene therapy applications), to detect hVR-1, hVR-2, and rVR-2 mRNA (*e.g.*, in a biological sample) or a genetic alteration in an hVR-1, hVR-2, and rVR-2 gene, and to modulate hVR-1, hVR-2, and rVR-2 activity, as described further below. The hVR-1, hVR-2, and rVR-2 proteins can be used to screen for naturally occurring hVR-1, hVR-2, and rVR-2 substrates, to screen for drugs or compounds which modulate
25 hVR-1, hVR-2, and rVR-2 activity, as well as to treat disorders characterized by insufficient or excessive production of hVR-1, hVR-2, and rVR-2 protein or production of hVR-1, hVR-2, and rVR-2 protein forms which have decreased or aberrant activity compared to hVR-1, hVR-2, and rVR-2 wild type protein (*e.g.*, pain disorders). Moreover, the anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies of the invention can
30 be used to detect and isolate hVR-1, hVR-2, and rVR-2 proteins, regulate the

bioavailability of hVR-1, hVR-2, and rVR-2 proteins, and modulate hVR-1, hVR-2, and rVR-2 activity.

A. Screening Assays:

5 The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to hVR-1, hVR-2, and rVR-2 proteins, have a stimulatory or inhibitory effect on, for example, hVR-1, hVR-2, and rVR-2 expression or hVR-1, hVR-2, and rVR-2 activity, or have a stimulatory or
10 inhibitory effect on, for example, the expression or activity of hVR-1, hVR-2, and rVR-2 substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of an hVR-1, hVR-2, and rVR-2 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides
15 assays for screening candidate or test compounds which bind to or modulate the activity of an hVR-1, hVR-2, and rVR-2 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries;
20 synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

25 Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in
30 Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage
5 (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell, *e.g.*, a neuronal cell, which expresses an hVR-1, hVR-2, and rVR-2 protein or biologically
10 active portion thereof is contacted with a test compound and the ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 activity is determined. Determining the ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 activity can be accomplished by monitoring, for example, intracellular calcium concentration or membrane depolarization by, *e.g.*, patch-clamp recordings in whole-cell, inside-out, and
15 outside-out configurations (as described in, for example, Tominaga M. *et al.* (1998) *Neuron* 21:531-543). Determining the ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 activity can further be accomplished by monitoring the activity of an hVR-1, hVR-2, and rVR-2-regulated transcription factor. The cell, for example, can be of mammalian origin, *e.g.*, a neuronal cell.

20 The ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 binding to a substrate or to bind to hVR-1, hVR-2, and rVR-2 can also be determined. Determining the ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 binding to a substrate can be accomplished, for example, by coupling the hVR-1, hVR-2, and rVR-2 substrate with a radioisotope or enzymatic label such that binding of the
25 hVR-1, hVR-2, and rVR-2 substrate to hVR-1, hVR-2, and rVR-2 can be determined by detecting the labeled hVR-1, hVR-2, and rVR-2 substrate in a complex. Determining the ability of the test compound to bind hVR-1, hVR-2, and rVR-2 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to hVR-1, hVR-2, and rVR-2 can be determined by detecting
30 the labeled hVR-1, hVR-2, and rVR-2 compound in a complex. For example, compounds (*e.g.*, hVR-1, hVR-2, and rVR-2 substrates) can be labeled with ¹²⁵I, ³⁵S,

^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, an hVR-1, hVR-2, and rVR-2 substrate) to interact with hVR-1, hVR-2, and rVR-2 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with hVR-1, hVR-2, and rVR-2 without the labeling of either the compound or the hVR-1, hVR-2, and rVR-2. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and hVR-1, hVR-2, and rVR-2.

In yet another embodiment, an assay of the present invention is a cell-free assay in which an hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is determined. biologically active portions of the hVR-1, hVR-2, and rVR-2 proteins to be used in assays of the present invention include fragments which participate in interactions with non-hVR-1, non-hVR-2, and non-rVR-2 molecules, *e.g.*, fragments with high surface probability scores. Binding of the test compound to the hVR-1, hVR-2, and rVR-2 protein can be determined either directly or indirectly as described above. In a embodiment, the assay includes contacting the hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof with a known compound which binds hVR-1, hVR-2, and rVR-2 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an hVR-1, hVR-2, and rVR-2 protein, wherein determining the ability of the test compound to interact with an hVR-1, hVR-2, and rVR-2 protein comprises determining the ability of the test

compound to preferentially bind to hVR-1, hVR-2, and rVR-2 or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which an hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of an hVR-1, hVR-2, and rVR-2 protein can be accomplished, for example, by determining the ability of the hVR-1, hVR-2, and rVR-2 protein to bind to an hVR-1, hVR-2, and rVR-2 target molecule, *e.g.*, a vanilloid compound such as capsaicin, by one of the methods described above for determining direct binding. Determining the ability of the hVR-1, hVR-2, and rVR-2 protein to bind to an hVR-1, hVR-2, and rVR-2 target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of an hVR-1, hVR-2, and rVR-2 protein can be accomplished by determining the ability of the hVR-1, hVR-2, and rVR-2 protein to further modulate the activity of a downstream effector of an hVR-1, hVR-2, and rVR-2 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting an hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof with a known compound which binds the hVR-1, hVR-2, and rVR-2 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the hVR-1, hVR-2, and rVR-2 protein, wherein

determining the ability of the test compound to interact with the hVR-1, hVR-2, and rVR-2 protein comprises determining the ability of the hVR-1, hVR-2, and rVR-2 protein to preferentially bind to or modulate the activity of an hVR-1, hVR-2, and rVR-2 target molecule.

- 5 The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of isolated proteins (*e.g.*, hVR-1, hVR-2, and rVR-2 proteins or biologically active portions thereof). In the case of cell-free assays in which a membrane-bound form of an isolated protein is used it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is
- 10 maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-
- 15 cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

- In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either hVR-1, hVR-2, and rVR-2 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or
- 20 both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to an hVR-1, hVR-2, and rVR-2 protein, or interaction of an hVR-1, hVR-2, and rVR-2 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-
- 25 centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ hVR-1, hVR-2, and rVR-2 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are
- 30 then combined with the test compound or the test compound and either the non-adsorbed target protein or hVR-1, hVR-2, and rVR-2 protein, and the mixture incubated under

conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, 5 the complexes can be dissociated from the matrix, and the level of hVR-1, hVR-2, and rVR-2 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either an hVR-1, hVR-2, and rVR-2 protein or an hVR-1, hVR-2, and rVR-2 target molecule can be immobilized utilizing 10 conjugation of biotin and streptavidin. Biotinylated hVR-1, hVR-2, and rVR-2 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with hVR-1, hVR-2, and rVR-2 protein or target 15 molecules but which do not interfere with binding of the hVR-1, hVR-2, and rVR-2 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or hVR-1, hVR-2, and rVR-2 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes 20 using antibodies reactive with the hVR-1, hVR-2, and rVR-2 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the hVR-1, hVR-2, and rVR-2 protein or target molecule.

In another embodiment, modulators of hVR-1, hVR-2, and rVR-2 expression are identified in a method wherein a cell is contacted with a candidate compound and the 25 expression of hVR-1, hVR-2, and rVR-2 mRNA or protein in the cell is determined. The level of expression of hVR-1, hVR-2, and rVR-2 mRNA or protein in the presence of the candidate compound is compared to the level of expression of hVR-1, hVR-2, and rVR-2 mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of hVR-1, hVR-2, and rVR-2 30 expression based on this comparison. For example, when expression of hVR-1, hVR-2, and rVR-2 mRNA or protein is greater (statistically significantly greater) in the presence

of the candidate compound than in its absence, the candidate compound is identified as a stimulator of hVR-1, hVR-2, and rVR-2 mRNA or protein expression. Alternatively, when expression of hVR-1, hVR-2, and rVR-2 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of hVR-1, hVR-2, and rVR-2 mRNA or protein expression. The level of hVR-1, hVR-2, and rVR-2 mRNA or protein expression in the cells can be determined by methods described herein for detecting hVR-1, hVR-2, and rVR-2 mRNA or protein.

In yet another aspect of the invention, the hVR-1, hVR-2, and rVR-2 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with hVR-1, hVR-2, and rVR-2 ("hVR-1-binding proteins", "hVR-2-binding proteins", and "rVR-2-binding proteins" or "hVR-1-bp", "hVR-2-bp", and "rVR-2-bp") and are involved in hVR-1, hVR-2, and rVR-2 activity. Such hVR-1, hVR-2, and rVR-2-binding proteins are also likely to be involved in the propagation of signals by the hVR-1, hVR-2, and rVR-2 proteins or hVR-1, hVR-2, and rVR-2 targets as, for example, downstream elements of an hVR-1, hVR-2, and rVR-2-mediated signaling pathway, *e.g.*, a pain signaling pathway. Alternatively, such hVR-1, hVR-2, and rVR-2-binding proteins are likely to be hVR-1, hVR-2, and rVR-2 inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for an hVR-1, hVR-2, and rVR-2 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an hVR-1, hVR-2, and rVR-2-dependent complex, the DNA-binding and

activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription
5 factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the hVR-1, hVR-2, and rVR-2 protein.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an
10 agent identified as described herein (*e.g.*, an hVR-1, hVR-2, and rVR-2 modulating agent, an antisense hVR-1, hVR-2, and rVR-2 nucleic acid molecule, an hVR-1, hVR-2, and rVR-2-specific antibody, or an hVR-1, hVR-2, and rVR-2-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in
15 an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

20 Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue
25 typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this
30 sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the hVR-1, hVR-2,

and rVR-2 nucleotide sequences, described herein, can be used to map the location of the hVR-1, hVR-2, and rVR-2 genes on a chromosome. The mapping of the hVR-1, hVR-2, and rVR-2 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

5 Briefly, hVR-1, hVR-2, and rVR-2 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the hVR-1, hVR-2, and rVR-2 nucleotide sequences. Computer analysis of the hVR-1, hVR-2, and rVR-2 sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be
10 used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the hVR-1, hVR-2, and rVR-2 sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow
15 and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single
20 human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

25 PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the hVR-1, hVR-2, and rVR-2 nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which
30 can similarly be used to map an hVR-1, hVR-2, and rVR-2 sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci.*

USA, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the hVR-1, hVR-2, and rVR-2 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not

in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based
5 on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

10 The hVR-1, hVR-2, and rVR-2 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield
15 unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an
20 alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the hVR-1, hVR-2, and rVR-2 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

25 Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The hVR-1, hVR-2, and rVR-2 nucleotide sequences of the invention
30 uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding

regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur
5 in the noncoding regions, fewer sequences are necessary to differentiate individuals.

If a panel of reagents from hVR-1, hVR-2, and rVR-2 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead,
10 can be made from extremely small tissue samples.

3. Use of Partial hVR-1, hVR-2, and rVR-2 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence
15 found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the
20 origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular
25 individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Examples of polynucleotide reagents include the hVR-1, hVR-2, and rVR-2 nucleotide sequences or portions thereof, *e.g.*, fragments derived from SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 11 having a length of at least 20 bases, preferably at least
30 30 bases.

The hVR-1, hVR-2, and rVR-2 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such hVR-1, hVR-2, and rVR-2 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, hVR-1, hVR-2, and rVR-2 primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

10

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining hVR-1, hVR-2, and rVR-2 protein and/or nucleic acid expression as well as hVR-1, hVR-2, and rVR-2 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with hVR-1, hVR-2, and rVR-2 protein, nucleic acid expression or activity. For example, mutations in an hVR-1, hVR-2, and rVR-2 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with hVR-1, hVR-2, and rVR-2 protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of hVR-1, hVR-2, and rVR-2 in clinical trials.

These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of hVR-1, hVR-2, and rVR-2 protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting hVR-1, hVR-2, and rVR-2 protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes hVR-1, hVR-2, and rVR-2 protein such that the presence of hVR-1, hVR-2, and rVR-2 protein or nucleic acid is detected in the biological sample. A agent for detecting hVR-1, hVR-2, and rVR-2 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to hVR-1, hVR-2, and rVR-2 mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length hVR-1, hVR-2, and rVR-2 nucleic acid, such as the nucleic acid of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to hVR-1, hVR-2, and rVR-2 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting hVR-1, hVR-2, and rVR-2 protein is an antibody capable of binding to hVR-1, hVR-2, and rVR-2 protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect hVR-1, hVR-2, and rVR-2 mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of hVR-1, hVR-2, and rVR-2 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro*

techniques for detection of hVR-1, hVR-2, and rVR-2 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of hVR-1, hVR-2, and rVR-2 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for
5 detection of hVR-1, hVR-2, and rVR-2 protein include introducing into a subject a labeled anti-hVR-1, hVR-2, and rVR-2 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the
10 test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a
15 compound or agent capable of detecting hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA, such that the presence of hVR-1, hVR-2, and rVR-2 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of hVR-1, hVR-2, and rVR-2 protein, mRNA or genomic DNA in the control sample with the
20 presence of hVR-1, hVR-2, and rVR-2 protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of hVR-1, hVR-2, and rVR-2 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting hVR-1, hVR-2, and rVR-2 protein or mRNA in a biological sample; means for determining the amount of hVR-1, hVR-2, and rVR-2 in
25 the sample; and means for comparing the amount of hVR-1, hVR-2, and rVR-2 in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect hVR-1, hVR-2, and rVR-2 protein or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity. As used herein, the term "aberrant" includes an hVR-1, hVR-2, and rVR-2 expression or activity which deviates from the wild type hVR-1, hVR-2, and rVR-2 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant hVR-1, hVR-2, and rVR-2 expression or activity is intended to include the cases in which a mutation in the hVR-1, hVR-2, and rVR-2 gene causes the hVR-1, hVR-2, and rVR-2 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional hVR-1, hVR-2, and rVR-2 protein or a protein which does not function in a wild-type fashion, *e.g.*, a protein which does not interact with an hVR-1, hVR-2, and rVR-2 ligand or one which interacts with a non-hVR-1, non-hVR-2, and non-rVR-2 ligand.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in hVR-1, hVR-2, and rVR-2 protein activity or nucleic acid expression, such as a pain disorder. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in hVR-1, hVR-2, and rVR-2 protein activity or nucleic acid expression, such as a pain disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity in which a test sample is obtained from a subject and hVR-1, hVR-2, and rVR-2 protein or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of hVR-1, hVR-2, and rVR-2 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a pain disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity in which a test sample is obtained and hVR-1, hVR-2, and rVR-2 protein or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of hVR-1, hVR-2, and rVR-2 protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity).

The methods of the invention can also be used to detect genetic alterations in an hVR-1, hVR-2, and rVR-2 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in hVR-1, hVR-2, and rVR-2 protein activity or nucleic acid expression, such as a neurodegenerative disorder. In embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding an hVR-1, hVR-2, and rVR-2-protein, or the mis-expression of the hVR-1, hVR-2, and rVR-2 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from an hVR-1, hVR-2, and rVR-2 gene; 2) an addition of one or more nucleotides to an hVR-1, hVR-2, and rVR-2 gene; 3) a substitution of one or more nucleotides of an hVR-1, hVR-2, and rVR-2 gene; 4) a chromosomal rearrangement of an hVR-1, hVR-2, and rVR-2 gene; 5) an alteration in the level of a messenger RNA transcript of an hVR-1, hVR-2, and rVR-2 gene; 6) aberrant modification of an hVR-1, hVR-2, and rVR-2 gene, such as of the methylation pattern of the genomic DNA; 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of an hVR-1, hVR-2, and rVR-2 gene; 8) a non-wild type level of an hVR-1, hVR-2, and rVR-2-protein; 9) allelic loss of an hVR-1, hVR-2, and rVR-2 gene,

and 10) inappropriate post-translational modification of an hVR-1, hVR-2, and rVR-2-protein. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in an hVR-1, hVR-2, and rVR-2 gene. A biological sample is a tissue or serum sample isolated by conventional means from a subject.

5 In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which
10 can be particularly useful for detecting point mutations in the hVR-1, hVR-2, and rVR-2-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to an hVR-1, hVR-2, and
15 rVR-2 gene under conditions such that hybridization and amplification of the hVR-1, hVR-2, and rVR-2-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used
20 for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any
25 other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an hVR-1, hVR-2, and rVR-2 gene
30 from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally),

digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in hVR-1, hVR-2, and rVR-2 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in hVR-1, hVR-2, and rVR-2 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the hVR-1, hVR-2, and rVR-2 gene and detect mutations by comparing the sequence of the sample hVR-1, hVR-2, and rVR-2 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the hVR-1, hVR-2, and rVR-2 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing
5 heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type hVR-1, hVR-2, and rVR-2 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA
10 duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing
15 polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called
20 "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in hVR-1, hVR-2, and rVR-2 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based
25 on an hVR-1, hVR-2, and rVR-2 sequence, *e.g.*, a wild-type hVR-1, hVR-2, and rVR-2 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in hVR-1, hVR-2, and rVR-2 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control hVR-1, hVR-2, and rVR-2 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In an embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different

mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention.

- 5 Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an hVR-1, hVR-2, and rVR-2 gene.

Furthermore, any cell type or tissue in which hVR-1, hVR-2, and rVR-2 is expressed may be utilized in the prognostic assays described herein.

25 3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of an hVR-1, hVR-2, and rVR-2 protein can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase hVR-1, hVR-2, and rVR-2 gene expression, protein levels, or upregulate hVR-1, hVR-2, and rVR-2 activity, can be monitored in clinical trials of subjects exhibiting decreased hVR-1, hVR-2, and rVR-2

gene expression, protein levels, or downregulated hVR-1, hVR-2, and rVR-2 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease hVR-1, hVR-2, and rVR-2 gene expression, protein levels, or downregulate hVR-1, hVR-2, and rVR-2 activity, can be monitored in clinical trials of subjects exhibiting
5 increased hVR-1, hVR-2, and rVR-2 gene expression, protein levels, or upregulated hVR-1, hVR-2, and rVR-2 activity. In such clinical trials, the expression or activity of an hVR-1, hVR-2, and rVR-2 gene, and preferably, other genes that have been implicated in, for example, an hVR-1, hVR-2, and rVR-2-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

10 For example, and not by way of limitation, genes, including hVR-1, hVR-2, and rVR-2, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates hVR-1, hVR-2, and rVR-2 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on hVR-1, hVR-2, and rVR-2-associated disorders (*e.g.*, pain disorders), for
15 example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of hVR-1, hVR-2, and rVR-2 and other genes implicated in the hVR-1, hVR-2, and rVR-2-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein
20 produced, by one of the methods as described herein, or by measuring the levels of activity of hVR-1, hVR-2, and rVR-2 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

25 In an embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii)
30 detecting the level of expression of an hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-

administration samples from the subject; (iv) detecting the level of expression or activity of the hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA in the pre-administration sample with the hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of hVR-1, hVR-2, and rVR-2 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of hVR-1, hVR-2, and rVR-2 to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, hVR-1, hVR-2, and rVR-2 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the hVR-1, hVR-2, and rVR-2 molecules of the present invention or hVR-1, hVR-2, and rVR-2 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or

therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. Prophylactic Methods

5 In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant hVR-1, hVR-2, and rVR-2 expression or activity, by administering to the subject an hVR-1, hVR-2, and rVR-2 or an agent which modulates hVR-1, hVR-2, and rVR-2 expression or at least one hVR-1, hVR-2, and rVR-2 activity. Subjects at risk for a disease which is caused or contributed to by
10 aberrant hVR-1, hVR-2, and rVR-2 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the hVR-1, hVR-2, and rVR-2 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of hVR-
15 1, hVR-2, and rVR-2 aberrancy, for example, an hVR-1, hVR-2, and rVR-2, hVR-1, hVR-2, and rVR-2 agonist or hVR-1, hVR-2, and rVR-2 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

20 2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating hVR-1, hVR-2, and rVR-2 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with an hVR-1, hVR-2, and rVR-2 or agent that modulates one or more of the
25 activities of hVR-1, hVR-2, and rVR-2 protein activity associated with the cell. An agent that modulates hVR-1, hVR-2, and rVR-2 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of an hVR-1, hVR-2, and rVR-2 protein (*e.g.*, an hVR-1, hVR-2, and rVR-2 substrate), an hVR-1, hVR-2, and rVR-2 antibody, an hVR-1, hVR-2, and rVR-2 agonist
30 or antagonist, a peptidomimetic of an hVR-1, hVR-2, and rVR-2 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more hVR-1,

hVR-2, and rVR-2 activities. Examples of such stimulatory agents include active hVR-1, hVR-2, and rVR-2 protein and a nucleic acid molecule encoding hVR-1, hVR-2, and rVR-2 that has been introduced into the cell. In another embodiment, the agent inhibits one or more hVR-1, hVR-2, and rVR-2 activities. Examples of such inhibitory agents
5 include antisense hVR-1, hVR-2, and rVR-2 nucleic acid molecules, anti-hVR-1, hVR-2, and rVR-2 antibodies, and hVR-1, hVR-2, and rVR-2 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder
10 characterized by aberrant expression or activity of an hVR-1, hVR-2, and rVR-2 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) hVR-1, hVR-2, and rVR-2 expression or activity. In another embodiment, the method involves administering an
15 hVR-1, hVR-2, and rVR-2 protein or nucleic acid molecule as therapy to compensate for reduced or aberrant hVR-1, hVR-2, and rVR-2 expression or activity.

Stimulation of hVR-1, hVR-2, and rVR-2 activity is desirable in situations in which hVR-1, hVR-2, and rVR-2 is abnormally downregulated and/or in which increased hVR-1, hVR-2, and rVR-2 activity is likely to have a beneficial effect. For
20 example, stimulation of hVR-1, hVR-2, and rVR-2 activity is desirable in situations in which an hVR-1, hVR-2, and rVR-2 is downregulated and/or in which increased hVR-1, hVR-2, and rVR-2 activity is likely to have a beneficial effect. Likewise, inhibition of hVR-1, hVR-2, and rVR-2 activity is desirable in situations in which hVR-1, hVR-2, and rVR-2 is abnormally upregulated and/or in which decreased hVR-1, hVR-2, and
25 rVR-2 activity is likely to have a beneficial effect.

3. Pharmacogenomics

The hVR-1, hVR-2, and rVR-2 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on hVR-1, hVR-2,
30 and rVR-2 activity (e.g., hVR-1, hVR-2, and rVR-2 gene expression) as identified by a screening assay described herein can be administered to individuals to treat

(prophylactically or therapeutically) hVR-1, hVR-2, and rVR-2-associated disorders (e.g., pain disorders) associated with aberrant hVR-1, hVR-2, and rVR-2 activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer an hVR-1, hVR-2, and rVR-2 molecule or hVR-1, hVR-2, and rVR-2 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with an hVR-1, hVR-2, and rVR-2 molecule or hVR-1, hVR-2, and rVR-2 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11):983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically

significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a

5 "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their

10 individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that

15 encodes a drug's target is known (*e.g.*, an hVR-1, hVR-2, and rVR-2 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a

20 major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug.

25 These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently

30 experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as

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demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

5 Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, an hVR-1, hVR-2, and rVR-2 molecule or hVR-1, hVR-2, and rVR-2 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

10 Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an hVR-1,
15 hVR-2, and rVR-2 molecule or hVR-1, hVR-2, and rVR-2 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent
20 applications cited throughout this application, as well as the Figures and the Sequence Listing are incorporated herein by reference.

EXAMPLES

25 **EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF hVR-1, hVR-2, and rVR-2 cDNA**

In this example, the identification and characterization of the genes encoding hVR-1 (clone Fchrb87a6), hVR-2 (clone fh21e11), hVR-2 alternate form (clone fhob12c4), and rVR-2 (clone flrxbl47g11) are described.

30

Isolation of the hVR-1, hVR-2, and the rVR-2 cDNA

The invention is based, at least in part, on the discovery of two human genes and one rat gene encoding novel members of the Capsaicin/Vanilloid receptor family, referred to herein as hVR-1, hVR-2, and rVR-2, respectively. These clones were
5 identified from a human heart library and a rat dorsal root ganglion (DRG) library, based on sequence homology to the known rat VR-1 (Accession Number AF029310). The sequence of the two human clones and the rat clone was determined and found to contain open reading frames.

The nucleotide sequence of the full length hVR-1 cDNA and the predicted amino
10 acid sequence of the hVR-1 polypeptide are shown in Figure 1 and in SEQ ID NOs:1 and 2, respectively.

The nucleotide sequence of the full length hVR-2 cDNA and the predicted amino acid sequence of the hVR-2 polypeptide are shown in Figure 2 and in SEQ ID NOs:4 and 5, respectively.

15 The nucleotide sequence of the partial hVR-2 (alternate form) cDNA and the predicted amino acid sequence of the hVR-2 (alternate form) polypeptide are shown in Figure 3 and in SEQ ID NOs:7 and 8, respectively.

The amino acid sequence of the predicted full length human VR-2 protein (alternate form) is shown in Figure 16 and in SEQ ID NO:20.

20 The nucleotide sequence of the partial rVR-2 cDNA and the predicted amino acid sequence of the rVR-2 polypeptide are shown in Figure 4 and in SEQ ID NOs:10 and 11, respectively.

Analysis of the hVR-1, hVR-2, and rVR-2 Molecules

25 The hVR-1 protein (SEQ ID NO:2) was aligned with the human VR-2 protein (SEQ ID NO:5) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4. The results showed a 46.348% identity and 55.378% similarity between the two sequences (see Figure 5).

The hVR-1 nucleotide sequence (SEQ ID NO:1) was aligned with the human
30 VR-2 nucleotide sequence (SEQ ID NO:4) using the GAP program in the GCG software package (nwsgapdna matrix) and a gap weight of 50 and a length weight of 3. The

results showed a 55.316% identity and 55.316% similarity between the two sequences (see Figure 6).

The hVR-2 protein (SEQ ID NO:5) was aligned with the rat VR-2 protein (SEQ ID NO:11) using the CLUSTAL W (1.74) multiple sequence alignment program (Figure 7), as well as using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4. The results showed a 79.167% identity and 81.703% similarity between the two sequences (see Figure 8).

The hVR-1 nucleotide sequence (SEQ ID NO:1) was aligned with the rat VR-1 nucleotide sequence (Accession Number:AF029310) using the GAP program in the GCG software package (nws gapdna matrix) and a gap weight of 50 and a length weight of 3. The results showed a 82.125% identity and 82.125% similarity between the two sequences (see Figure 9).

The hVR-1 protein (SEQ ID NO:2) was aligned with the rat VR-1 protein (Accession Number:AF029310) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4. The results showed a 86.022% identity and 89.247% similarity between the two sequences (see Figure 10).

The hVR-2 protein (SEQ ID NO:5) was aligned with the human VR-2 protein (alternate form) (SEQ ID NO:8) using the CLUSTAL W (1.74) multiple sequence alignment program (Figure 11).

Finally, the hVR-2 protein (SEQ ID NO:5) was aligned with the predicted full length human VR-2 protein (alternate form) (SEQ ID NO:20) using the CLUSTAL W (1.74) multiple sequence alignment program (Figure 17).

A search was performed against the HMM database resulting in the identification of three ankyrin repeat domains in the amino acid sequence of hVR-1 (SEQ ID NO:2) at about residues 201-233, 248-283, and 333-361, and in the amino acid sequence of hVR-2 (SEQ ID NO:5) at about residues 162-194, 208-243, and 293-328. The results of the searches are set forth in Figures 13 and 15, respectively.

Hydropathy plots have identified 6 transmembrane domains in the hVR-1 and the hVR-2 proteins (see Figures 12 and 14, respectively).

A series of searches have revealed that the hVR-1 protein matches the ProDom entry 141801 for the vanilloid receptor subtype and the ProDom entry 145518 for the vanilloid receptor subtype.

Moreover, a search was performed against the Prosite database resulting in the
5 identification of four N-glycosylation sites in the amino acid sequence of SEQ ID NO:5 (at about residues 171-174, 192-195, 604-607, and 749-752), three cGMP-dependent protein kinase phosphorylation sites in the amino acid sequence of SEQ ID NO:5 (at about residues 2-5, 368-371, and 499-502), a series of protein kinase C and Casein kinase II phosphorylation sites in the amino acid sequence of SEQ ID NO:5, two
10 tyrosine kinase phosphorylation sites in the amino acid sequence of SEQ ID NO:5 (at about residues 368-375 and 622-628), and two myristoylation sites in the amino acid sequence of SEQ ID NO:5 (at about residues 169-174 and 765-770).

Tissue Distribution of hVR-1 and hVR-2 mRNA

15 This Example describes the tissue distribution of hVR-1 and hVR-2 mRNA as determined by *in situ* hybridization.

For *in situ* analysis, tissues, such as brain regions and whole brain, obtained from human and monkey were first frozen on dry ice. Ten-micrometer-thick coronal sections of the tissues were postfixed with 4% formaldehyde in DEPC treated 1X phosphate-
20 buffered saline at room temperature for 10 minutes before being rinsed twice in DEPC 1X phosphate-buffered saline and once in 0.1 M triethanolamine-HCl (pH 8.0). Following incubation in 0.25% acetic anhydride-0.1 M triethanolamine-HCl for 10 minutes, sections were rinsed in DEPC 2X SSC (1X SSC is 0.15M NaCl plus 0.015M sodium citrate). Tissue was then dehydrated through a series of ethanol washes,
25 incubated in 100% chloroform for 5 minutes, and then rinsed in 100% ethanol for 1 minute and 95% ethanol for 1 minute and allowed to air dry.

Hybridizations were performed with ³⁵S-radiolabeled (5 X 10⁷ cpm/ml) cRNA probes. Probes were incubated in the presence of a solution containing 600 mM NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 0.01% sheared salmon sperm DNA, 0.01% yeast
30 tRNA, 0.05% yeast total RNA type X1, 1 X Denhardt's solution, 50% formamide, 10%

dextran sulfate, 100 mM dithiothreitol, 0.1% sodium dodecyl sulfate (SDS), and 0.1% sodium thiosulfate for 18 hours at 55°C.

After hybridization, slides were washed with 2 X SSC. Sections were then sequentially incubated at 37°C in TNE (a solution containing 10 mM Tris-HCl (pH 7.6), 500 mM NaCl, and 1 mM EDTA), for 10 minutes, in TNE with 10µg of RNase A per ml for 30 minutes, and finally in TNE for 10 minutes. Slides were then rinsed with 2 X SSC at room temperature, washed with 2 X SSC at 50°C for 1 hour, washed with 0.2 X SSC at 55°C for 1 hour, and 0.2 X SSC at 60°C for 1 hour. Sections were then dehydrated rapidly through serial ethanol-0.3 M sodium acetate concentrations before being air dried and exposed to Kodak Biomax MR scientific imaging film for 24 hours and subsequently dipped in NB-2 photoemulsion and exposed at 4°C for 7 days before being developed and counter stained.

The data indicate that the hVR-1 molecule is not expressed in human nor monkey brain. The hVR-1 molecule is expressed in nodose, trigeminal sensory neurons, but is not expressed in sympathetic neurons. Within the nodose sensory neurons and trigeminal sensory neurons, expression was seen in distinct sub-populations. Moreover, hVR1 is expressed in some, but not all, small dorsal root ganglion (DRG) neurons and in a few medium sized DRG neurons. The hVR-1 molecule is partially co-expressed with the neuropeptide CGRP and with substance P which are present in nociceptive neurons.

The data further indicate that the VR-2 molecule is expressed in both human and monkey brain, primarily in cortical neurons. The VR2 molecule is also expressed in other brain regions, for example, the thalamus, striatum, hippocampus, hypothalamus, midbrain, medula and brain stem. In addition, the VR-2 molecule is expressed in parasympathetic neurons of the monkey heart (atrium), nodose sensory neurons, trigeminal (TRG) sensory neurons, dorsal root ganglion sensory neurons, sympathetic neurons, and motor neurons of the spinal cord. The VR2 molecule is widely expressed in TRG and DRG neurons, being present in most small and medium sized neurons and also in a few of the large neurons. VR2, like VR-1, partially co-localizes with CGRP and substance P.

Trigeminal sensory neurons are recognized pain centers while sympathetic neurons have been shown to be involved in neuropathic pain.

**EXAMPLE 2: EXPRESSION OF RECOMBINANT hVR-1, hVR-2, AND
rVR-2 PROTEIN IN BACTERIAL CELLS**

In this example, hVR-1, hVR-2, and rVR-2 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, hVR-1, hVR-2, and rVR-2 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-hVR-1, GST-hVR-2, and GST-rVR-2 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

**EXAMPLE 3: EXPRESSION OF RECOMBINANT hVR-1, hVR-2,
AND rVR-2 PROTEIN IN COS CELLS**

To express the hVR-1, hVR-2, and rVR-2 gene in COS cells, the pcDNA/Amp vector from Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire hVR-1, hVR-2, and rVR-2 protein and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the hVR-1, hVR-2, and rVR-2 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the hVR-1, hVR-2, and rVR-2 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop

codon, the HA tag or FLAG tag and the last 20 nucleotides of the hVR-1, hVR-2, and rVR-2 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two
5 restriction sites chosen are different so that the hVR-1, hVR-2, and rVR-2 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5a, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and
10 examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the hVR-1, hVR-2, and rVR-2-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in
15 Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the hVR-1, hVR-2, and rVR-2 polypeptide is detected by radiolabelling (³⁵S-methionine or ³⁵S-cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D.
20 *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labelled for 8 hours with ³⁵S-methionine (or ³⁵S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture
25 media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the hVR-1, hVR-2, and rVR-2 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner
30 described above, and the expression of the hVR-1, hVR-2, and rVR-2 polypeptide is

detected by radiolabelling and immunoprecipitation using an hVR-1, hVR-2, and rVR-2 specific monoclonal antibody.

EXAMPLE 4: ELECTROPHYSIOLOGICAL STUDIES OF VR2

5 Human VR2 was functionally characterized in both HEK293 cells and *Xenopus* oocytes using electrophysiological methods. VR2 (in the pcDNA3.1 vector purchased by Invitrogen) was transiently expressed in HEK293 cells (ATCC) and recordings were performed 48 hours after transfection of cells using the whole-cell patch-clamp method (described in Bertil Hille, *Ionin Channels of excitable membranes*, 1992; Hammill *et al.* 10 (1981) *Pluger Arch.* 391:85-100). The results indicate that heat stimulation (>50 °C) induces a rapid inactivating inward current (1-2 nA). Heat-evoked currents of VR2 displayed profound desensitization and could be reversibly blocked by the VR1 inhibitor capsazepin (at a 10 µM concentration). In contrast to rat VR1, Capsaicin (at a 1-10 µM concentration), resiniferatoxin (at a 0.1-3 µM concentration), and low pH (5.0-6.0) do 15 not induce any currents from VR2. Binding studies of [³H]-resiniferatoxin (NEN) to both human VR1 and VR2 in membranes isolated from HEK293 cell homogenates also indicate that resiniferatoxin (at a 0.1-10 nM concentration) has no specific binding to VR2 while it binds to human VR1 with high affinities.

For the oocyte studies, human VR2 was subcloned into an oocyte expression 20 vector containing 5'- and 3'-UTR of *Xenopus* β-globin (Chiara *et al.* (1999) *Biochemistry* 38(20)6689-6698). *In vitro* trasncription was carried out as described in Chiara *et al.* (*supra*) and cRNA (10-100 ng) was then injected into the oocytes. VR2 function was characterized in the oocytes 48 hours after cRNA injection using a standard two-electrode voltage-clamp. Consistent with the data from the HEK293 studies, VR2 25 can only be activated by heat stimulation (48-50 °C) but not by vanilloid receptor agonists, capsaicin, or resiniferatoxin. The vanilloid receptor antagonist capsazepine (at a 1-10 µM concentration) blocks the heat response of VR2 reversibly.

EXAMPLE 5: GENERATION OF ANTI-hVR-2 ANTIBODIES AND hVR-2 PROTEIN LOCALIZATION BY IMMUNOSTAINING

Polyclonal antisera were raised in rabbits against the following three peptides derived from the human VR2 amino acid sequence, using the techniques described in Ed Harlow and David Lane (1988) "Antibodies; A Laboratory Manual" Cold Spring harbor Laboratory Press.

Antibody PEPTIDE 1: AFHCKSPHRHRMVVLE (SEQ ID NO:13)

Antibody PEPTIDE 2: RPEAPTGPNATESVQPMEGQEDEGN (SEQ ID NO:14)

Antibody PEPTIDE 3: SVLEMENGYWWCRKKQRAG (SEQ ID NO:15)

10 Antisera were subsequently affinity purified using the peptide immunogen.

The polyclonal antisera were tested for immunostaining of both monkey and rat dorsal root ganglion sensory neurons. Peptides 1 and 3 gave specific staining of subpopulations of sensory neurons that was competed with the corresponding peptide. This pattern of expression was very similar to the one observed using a VR-2 riboprobe.

15

EXAMPLE 6: CHROMOSOMAL LOCALIZATION OF hVR-1 AND hVR-2

To chromosomally map the hVR-1 gene, primers were designed based on the sequence of hVR-1 (clone Fchr87a6) (amplifying a 177 bp product from a human control cell line DNA and multiple faint larger products from a control Hamster cell line DNA by PCR). These primers were used to amplify 93 DNAs in duplicate from the Genebridge 4 Radiation Hybrid Panel (Research Genetics, Inc., Huntsville, AL).

The hVR-1 primers used in the PCR mapping studies were: forward - TAGGAGACCCCGTTGCCACG (SEQ ID NO:16) and reverse - GATTCACCTGGGGACAGTGACG (SEQ ID NO:17) and the PCR reactions were performed as follows: 5 µl Template DNA (10ng/µl), 1.5µl 10X Perkin Elmer PCR Buffer, 1.2µl Pharmacia dNTP mix 2.5 mM, 1.15µl Forward primer 6.6µM, 1.15µl Reverse primer 6.6µM, 5µl Gibco/BRL Platinum Taq .05U/µl (Hot Start), using an amplification profile of: 95°C for 10 minutes followed by 35 Cycles of 94°C for 40 seconds, 55°C for 40 seconds, 72°C for 40 seconds, and 72°C for 5 minutes. The PCR

products were run on 2% agarose gels, post-stained with SYBR Gold (1:10,000 dilution in 1X TBE), and scanned on a Molecular Dynamics 595 Fluorimager.

The following is the vector data for the 93 Genebridge4 hybrid DNAs. These are in order 1-93. A "1" is a positive result, a "-" is a negative result, a "?" is an ambiguous result.

```
hVR1  1 - - 1 ? - 1 - 1 - 1 1 - - - 1 - - 1 - 1 1 - - 1 - 1 - 1 - - 1 1 - 1 - - - - - 1 - 1 1 1 - - -
1 - - - 1 1 - - - - 1 - - - - 1 - 1 - - 1 1 1 - 1 - 1 - - - 1 1 - 1 - - - - - 1
```

10 RH linkage analysis was performed using the Map Manager QTb28 software package.

hVR1 was found to map to the p arm of human chromosome 17, 18.9 cR₃₀₀₀ telomeric to the Whitehead Institute framework marker WI-6584, and 7.7 cR₃₀₀₀ centromeric of the Whitehead framework marker WI-5436. LOD scores for linkage were 14.5 for WI-6584 and 19.3 for WI-5436. This region corresponds to the

15 cytogenetic location 17p12-13. This region is syntenic to mouse chromosome 11.

To chromosomally map the hVR-2 gene, primers were designed from 5' UTR sequence of human VR2 (clone Flh21e11) (amplifying a 166 bp product from a human control cell line DNA and 2 much larger faint bands from a control Hamster cell line DNA by PCR). These primers were used to amplify 93 DNAs in duplicate from the

20 Genebridge 4 Radiation Hybrid Panel (Research Genetics, Inc., Huntsville, AL).

The hVR-2 primers used in the PCR mapping studies were: forward - TTAAGCTCCCGTTCTCACCG (SEQ ID NO:18) and reverse -

GCTGCGGGAGGAAGTGAAGC (SEQ ID NO:19) and the PCR reactions were performed as follows: 5µl Template DNA (10ng/µl), 1.5µl 10X Perkin Elmer PCR

25 Buffer, 1.2µl Pharmacia dNTP mix 2.5mM, 1.15µl Forward primer 6.6µM, 1.15µl Reverse primer 6.6µM, 5µl Gibco/BRL Platinum Taq .05U/µl (Hot Start), using an amplification profile of 95°C for 10 minutes, followed by 35 Cycles of 94°C for 40

seconds, 55°C for 40 seconds, 72°C for 40 seconds, and 72°C for 5 minutes. The PCR products were run on 2% agarose gels, post-stained with SYBR Gold (1:10,000 dilution

30 in 1X TBE), and scanned on a Molecular Dynamics 595 Fluorimager.

The following is the vector data for the 93 Genebridge4 hybrid DNAs. These are in order 1-93. A "1" is a positive result, a "-" is a negative result, a "?" is an ambiguous result.

5 hVR2 1 - - 1 1 - ? 1 1 - - - - - 1 - - 1 - 1 1 - - - 1 - 1 1 1 1 - - 1 - - - - - 1 1 - 1 1 1 - - 1
1 - - - 1 1 - - - - 1 1 1 - - - 1 - 1 1 - 1 - 1 1 1 1 1 - - - 1 1 - 1 - 1 - ? - - - ?

RH linkage analysis was performed using the Map Manager QTb28 software package.

10 hVR2 was found to map to the p arm of human chromosome 17, 29.3cR cR₃₀₀₀ telomeric to the Whitehead Institute framework marker D17S721, and 23.3 cR₃₀₀₀ centromeric of the Whitehead framework marker AFMA043ZB5. LOD scores for linkage were 11.9 for D17S721 and 13.6 for AFMA043ZB5. This region corresponds to the cytogenetic location 17p11-12. This region is syntenic to mouse chromosome 11.

15

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

20 claims.

What is claimed:

1. An isolated nucleic acid molecule selected from the group consisting of:
 - (a) a nucleic acid molecule comprising the nucleotide sequence set
5 forth in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 or a complement thereof; and
 - (b) a nucleic acid molecule consisting of the nucleotide sequence set
forth in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 or a complement thereof.
- 10 2. An isolated nucleic acid molecule which encodes a polypeptide selected
from the group consisting of:
 - (a) a polypeptide comprising the amino acid sequence set forth in
SEQ ID NO:2, 5, 8, or 11; and
 - (b) a polypeptide consisting of the amino acid sequence set forth in
SEQ ID NO:2, 5, 8, or 11.
- 15 3. An isolated nucleic acid molecule which encodes a naturally occurring
allelic variant of a polypeptide comprising the amino acid sequence set forth in SEQ ID
NO:2, 5, 8, or 11.
- 20 4. An isolated nucleic acid molecule selected from the group consisting of:
 - a) a nucleic acid molecule comprising a nucleotide sequence which
is at least 83% identical to the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or
12, or a complement thereof;
 - b) a nucleic acid molecule comprising a fragment of at least 20
25 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, 3, 4,
6, 7, 9, 10, or 12, or a complement thereof;
 - c) a nucleic acid molecule which encodes a polypeptide comprising
an amino acid sequence at least about 87% identical to the amino acid sequence of SEQ
ID NO:2, 5, 8, or 11; and

- 100 -

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the fragment comprises at least 15 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2, 5, 8, or 11.

5

5. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1, 2, 3, or 4, and a nucleotide sequence encoding a heterologous polypeptide.

10

6. A vector comprising the nucleic acid molecule of any one of claims 1, 2, 3, or 4.

7. The vector of claim 6, which is an expression vector.

15

8. A host cell transfected with the expression vector of claim 7.

9. A method of expressing a polypeptide comprising culturing the host cell of claim 8 in an appropriate culture medium to, thereby, express the polypeptide.

10. An isolated polypeptide selected from the group consisting of:
- a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, 5, 8, or 11;
 - 5 b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule consisting of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 under stringent conditions;
 - c) a polypeptide which is encoded by a nucleic acid molecule
10 comprising a nucleotide sequence which is at least 83% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12; and
 - d) a polypeptide comprising an amino acid sequence which is at least 87% identical to the amino acid sequence of SEQ ID NO:2, 5, 8, or 11.
- 15 11. The isolated polypeptide of claim 10 comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11.
12. The polypeptide of claim 10, further comprising heterologous amino acid sequences.
- 20 13. An antibody which selectively binds to a polypeptide of claim 10.
14. A method for detecting the presence of a polypeptide of claim 10 in a sample comprising:
- 25 a) contacting the sample with a compound which selectively binds to the polypeptide; and
 - b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 10 in the sample.
- 30 15. The method of claim 14, wherein the compound which binds to the polypeptide is an antibody.

16. A kit comprising a compound which selectively binds to a polypeptide of claim 10 and instructions for use.

5 17. A method for detecting the presence of a nucleic acid molecule of any one of claims 1, 2, 3, or 4 in a sample comprising:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to a
10 nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of any one of claims 1, 2, 3, or 4 in the sample.

18. The method of claim 17, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

15

19. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of any one of claims 1, 2, 3, or 4 and instructions for use.

20. A method for identifying a compound which binds to a polypeptide of
20 claim 10 comprising:

a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and

b) determining whether the polypeptide binds to the test compound.

25 21. The method of claim 20, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detection of test
compound/polypeptide binding;

b) detection of binding using a competition binding assay; and

30 c) detection of binding using an assay for hVR-1, hVR-2, or rVR-2 activity.

22. A method for modulating the activity of a polypeptide of claim 10 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

5

23. A method for identifying a compound which modulates the activity of a polypeptide of claim 10 comprising:

- a) contacting a polypeptide of claim 10 with a test compound; and
 - b) determining the effect of the test compound on the activity of the
- 10 polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

24. A method for treating a subject having a disorder characterized by aberrant hVR-1 or hVR-2 protein activity or nucleic acid expression comprising

15 administering to the subject a hVR-1 or hVR-2 modulator such that treatment of the subject occurs.

25. The method of claim 24, wherein the hVR-1 or hVR-2 modulator is a small molecule.

20

26. The method of claim 24, wherein the disorder is a pain disorder.

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humanVR1 gene with translation of open reading frame

Input file Fchrb87a6.seq; Output File Fchrb87a6.tra
Sequence length 3909

GTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAA
 CGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGG
 GCAGTGAGCGCAACGCACTTGCAGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGC
 GCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACT
 GCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTG
 AGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCCTTTACAC
 TTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATT
 ACGCCAAGCTCTAATACGACTCACTATAGGGAAAGCTGGTACGCCTGCAGGTACCGGTCCGGAATTTCCGGGTCGACCC
 ACGCGTCCGAAACACACCTCTCTGCTGTGGAAGACTGTGCAATGGCACAGCCGCAGAGCTTGGTTTGGGAGGTTGAA
 GTGCTCTGGGGAGAATTCGTAGATCATCCTCAGAAAAGCCTTGCCCTGGTGTCTACCAGAAAAACGTCTCCCAATCAC
 CCAGAAAAGCTGTCCACAGTAGTCCCCCTTATCCACGGGTGTCACTTTCCATGGGTTTCAGTTATTTGCGGTCAACCAC
 GGTCTGCCAATATTAAATGGAAAATTCTTCAAACAGTTCCCAAGTTTCCCTTGTGCATTGTTCTGAGCAGTGTGATGA
 AGAGTCTCTGCCGTGCCATCTGGGATGCAAACCGTCCCTGTGTCCCCACGTCCAGGCCGTAGATGCTCCCCGCCGGTC
 AGTCACTTAGTCGTCAGATCGCCCGTCTGGTATCACAGTGCTTCTGTTTCAGGTTGCACACTGGGCCACAGAGGATCCA

 M K K W S S T D L G T A A D P L Q K 18
 GCAAGG ATG AAG AAA TGG AGC AGC ACA GAC TTG GGG ACA GCT GCG GAC CCA CTC CAA AAG 54

 D T C P D P L D G D P N S R P P P A K P 38
 GAC ACC TGC CCA GAC CCC CTG GAT GGA GAC CCT AAC TCC AGG CCA CCT CCA GCC AAG CCC 114

 Q L P T A K S R T R L F G K G D S E E A 58
 CAG CTC CCC ACG GCC AAG AGC CGC ACC CGG CTC TTT GGG AAG GGT GAC TCG GAG GAG GCT 174

 F P V D C P H E E G E L D S C P T I T V 78
 TTC CCG GTG GAT TGC CCC CAC GAG GAA GGT GAG TTG GAC TCC TGC CCG ACC ATC ACA GTC 234

 S P V I T I Q R P G D G P T G A R L L S 98
 AGC CCT GTT ATC ACC ATC CAG AGG CCA GGA GAC GGC CCC ACC GGT GCC AGG CTG CTG TCC 294

Fig. 1

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Q	D	S	V	A	A	S	T	E	K	T	L	R	L	Y	D	R	R	S	I	118
CAG	GAC	TCT	GTC	GCC	GCC	AGC	ACC	GAG	AAG	ACC	CTC	AGG	CTC	TAT	GAT	CGC	AGG	AGT	ATC	354
F	E	A	V	A	Q	N	N	C	Q	D	L	E	S	L	L	L	F	L	Q	138
TTT	GAA	GCC	GTT	GCT	CAG	AAT	AAC	TGC	CAG	GAT	CTG	GAG	AGC	CTG	CTG	CTC	TTC	CTG	CAG	414
K	S	K	K	H	L	T	D	N	E	F	K	D	P	E	T	G	K	T	C	158
AAG	AGC	AAG	AAG	CAC	CTC	ACA	GAC	AAC	GAG	TTC	AAA	GAC	CCT	GAG	ACA	GGG	AAG	ACC	TGT	474
L	L	K	A	M	L	N	L	H	D	G	Q	N	T	T	I	P	L	L	L	178
CTG	CTG	AAA	GCC	ATG	CTC	AAC	CTG	CAC	GAC	GGA	CAG	AAC	ACC	ACC	ATC	CCC	CTG	CTC	CTG	534
E	I	A	R	Q	T	D	S	L	K	E	L	V	N	A	S	Y	T	D	S	198
GAG	ATC	GCG	CGG	CAA	ACG	GAC	AGC	CTG	AAG	GAG	CTT	GTC	AAC	GCC	AGC	TAC	ACG	GAC	AGC	594
Y	Y	K	G	Q	T	A	L	H	I	A	I	E	R	R	N	M	A	L	V	218
TAC	TAC	AAG	GGC	CAG	ACA	GCA	CTG	CAC	ATC	GCC	ATC	GAG	AGA	CGC	AAC	ATG	GCC	CTG	GTG	654
T	L	L	V	E	N	G	A	D	V	Q	A	A	A	H	G	D	F	F	K	238
ACC	CTC	CTG	GTG	GAG	AAC	GGA	GCA	GAC	GTC	CAG	GCT	GCG	GCC	CAT	GGG	GAC	TTC	TTT	AAG	714
K	T	K	G	R	P	G	F	Y	F	G	E	L	P	L	S	L	A	A	C	258
AAA	ACC	AAA	GGG	CGG	CCT	GGA	TTC	TAC	TTC	GGT	GAA	CTG	CCC	CTG	TCC	CTG	GCC	GCG	TGC	774
T	N	Q	L	G	I	V	K	F	L	L	Q	N	S	W	Q	T	A	D	I	278
ACC	AAC	CAG	CTG	GGC	ATC	GTG	AAG	TTC	CTG	CTG	CAG	AAC	TCC	TGG	CAG	ACG	GCC	GAC	ATC	834
S	A	R	D	S	V	G	N	T	V	L	H	A	L	V	E	V	A	D	N	298
AGC	GCC	AGG	GAC	TCG	GTG	GGC	AAC	ACG	GTG	CTG	CAC	GCC	CTG	GTG	GAG	GTG	GCC	GAC	AAC	894
T	A	D	N	T	K	F	V	T	S	M	Y	N	E	I	L	M	L	G	A	318
ACG	GCC	GAC	AAC	ACG	AAG	TTT	GTG	ACG	AGC	ATG	TAC	AAT	GAG	ATT	CTG	ATG	CTG	GGG	GCC	954
K	L	H	P	T	L	K	L	E	E	L	T	N	K	K	G	M	T	P	L	338
AAA	CTG	CAC	CCG	ACG	CTG	AAG	CTG	GAG	GAG	CTC	ACC	AAC	AAG	AAG	GGA	ATG	ACG	CCG	CTG	1014
A	L	A	A	G	T	G	K	I	G	V	L	A	Y	I	L	Q	R	E	I	358
GCT	CTG	GCA	GCT	GGG	ACC	GGG	AAG	ATC	GGG	GTC	TTG	GCC	TAT	ATT	CTC	CAG	CGG	GAG	ATC	1074
Q	E	P	E	C	R	H	L	S	R	K	F	T	E	W	A	Y	G	P	V	378
CAG	GAG	CCC	GAG	TGC	AGG	CAC	CTG	TCC	AGG	AAG	TTC	ACC	GAG	TGG	GCC	TAC	GGG	CCC	GTG	1134
H	S	S	L	Y	D	L	S	C	I	D	T	C	E	K	N	S	V	L	E	398
CAC	TCC	TCG	CTG	TAC	GAC	CTG	TCC	TGC	ATC	GAC	ACC	TGC	GAG	AAG	AAC	TCG	GTG	CTG	GAG	1194
V	I	A	Y	S	S	S	E	T	P	N	R	H	D	M	L	L	V	E	P	418
GTG	ATC	GCC	TAC	AGC	AGC	AGC	GAG	ACC	CCT	AAT	CGC	CAC	GAC	ATG	CTC	TTG	GTG	GAG	CCG	1254

Fig. 1 continued

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L N R L L Q D K W D R F V K R I F Y F N 438
 CTG AAC CGA CTC CTG CAG GAC AAG TGG GAC AGA TTC GTC AAG CGC ATC TTC TAC TTC AAC 1314

 F L V Y C L Y M I I F T M A A Y Y R P V 458
 TTC CTG GTC TAC TGC CTG TAC ATG ATC ATC TTC ACC ATG GCT GCC TAC TAC AGG CCC GTG 1374

 D G L P P F K M E K I G D Y F R V T G E 478
 GAT GGC TTG CCT CCC TTT AAG ATG GAA ARA ATT GGA GAC TAT TTC CGA GTT ACT GGA GAG 1434

 I L S V L G G V Y F F F R G I Q Y F L Q 498
 ATC CTG TCT GTG TTA GGA GGA GTC TAC TTC TTT TTC CGA GGG ATT CAG TAT TTC CTG CAG 1494

 R R P S M K T L F V D S Y S E M L F F L 518
 AGG CGG CCG TCG ATG AAG ACC CTG TTT GTG GAC AGC TAC AGT GAG ATG CTT TTC TTT CTG 1554

 Q S L F M L A T V V L Y F S H L K E Y V 538
 CAG TCA CTG TTC ATG CTG GCC ACC GTG GTG CTG TAC TTC AGC CAC CTC AAG GAG TAT GTG 1614

 A S M V F S L A L G W T N M L Y Y T R G 558
 GCT TCC ATG GTA TTC TCC CTG GCC TTG GGC TGG ACC AAC ATG CTC TAC TAC ACC CGC GGT 1674

 F Q Q M G I Y A V M I E K M I L R D L C 578
 TTC CAG CAG ATG GGC ATC TAT GCC GTC ATG ATA GAG AAG ATG ATC CTG AGA GAC CTG TGC 1734

 R F M F V Y I V F L F G F S T A V V T L 598
 CGT TTC ATG TTT GTC TAC ATC GTC TTC TTG TTC GGG TTT TCC ACA GCG GTG GTG ACG CTG 1794

 I E D G K N D S L P S E S T S H R W R G 618
 ATT GAA GAC GGG AAG AAT GAC TCC CTG CCG TCT GAG TCC ACG TCG CAC AGG TGG CGG GGG 1854

 P A C R P P D S S Y N S L Y S T C L E L 638
 CCT GCC TGC AGG CCC CCC GAT AGC TCC TAC AAC AGC CTG TAC TCC ACC TGC CTG GAG CTG 1914

 F K F T I G M G D L E F T E N Y D F K A 658
 TTC AAG TTC ACC ATC GGC ATG GGC GAC CTG GAG TTC ACT GAG AAC TAT GAC TTC AAG GCT 1974

 V F I I L L L A Y V I L T Y I L L L N M 678
 GTC TTC ATC ATC CTG CTG CTG GCC TAT GTA ATT CTC ACC TAC ATC CTC CTG CTC AAC ATG 2034

 L I A L M G E T V N K I A Q E S K N I W 698
 CTC ATC GCC CTC ATG GGT GAG ACT GTC AAC AAG ATC GCA CAG GAG AGC AAG AAC ATC TGG 2094

 K L Q R A I T I L D T E K S F L K C M R 718
 AAG CTG CAG AGA GCC ATC ACC ATC CTG GAC ACG GAG AAG AGC TTC CTT AAG TGC ATG AGG 2154

Fig. 1 continued

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K A F R S G K L L Q V G Y T P D G K D D 738
AAG GCC TTC CGC TCA GGC AAG CTG CTG CAG GTG GGG TAC ACA CCT GAT GGC AAG GAC GAC 2214

Y R W C F R V D E V N W T T W N T N V G 758
TAC CGG TGG TGC TTC AGG GTG GAC GAG GTG AAC TGG ACC ACC TGG AAC ACC AAC GTG GGC 2274

I I N E D P G N C E G V K R T L S F S L 778
ATC ATC AAC GAA GAC CCG GGC AAC TGT GAG GGC GTC AAG CGC ACC CTG AGC TTC TCC CTG 2334

R S S R V S G R H W K N F A L V P L L R 798
CGG TCA AGC AGA GTT TCA GGC AGA CAC TGG AAG AAC TTT GCC CTG GTC CCC CTT TTA AGA 2394

E A S A R D R Q S A Q P E E V Y L R Q F 818
GAG GCA AGT GCT CGA GAT AGG CAG TCT GCT CAG CCC GAG GAA GTT TAT CTG CGA CAG TTT 2454

S G S L K P E D A E V F K S P A A S G E 838
TCA GGG TCT CTG AAG CCA GAG GAC GCT GAG GTC TTC AAG AGT CCT GCC GCT TCC GGG GAG 2514

K * 840
AAG TGA 2520

GGACGTCACGCAGACAGCACTGTCAACACTGGGCCTTAGGAGACCCCGTTGCCACGGGGGGCTGCTGAGGGAACACCAG
TGCTCTGTCAGCAGCCTGGCCTGGTCTGTGCCTGCCCAGCATGTTCCCAAATCTGTGCTGGACAAGCTGTGGGAAGCGT
TCTTGGAAGCATGGGGAGTGATGTACATCCAACCGTCACTGTCCCCAAGTGAATCTCCTAACAGACTTTCAGGTTTTTA
CTCACTTTACTAAAAAAAAAAAAAAAAAGGGCGGCCGCTTA

Fig. 1 continued

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Full-length human VR2

Input file Flh21e11.seq; Output File Flh21e11.tra

Sequence length 2809

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GGCTAGCCTGTCTGACAGGGGAGAGTTAAGCTCCCGTTCTCCACCGTGCCGGCTGGCCAGGTGGGCTGAGGGTGACCG
AGAGACCAGAACCTGCTTGCTGGAGCTTAGTGCTCAGAGCTGGGGAGGGAGGTTCCGCCGCTCCTCTGCTGTCAGCGCC
GGCAGCCCCCTCCCGGCTTCACTTCCTCCCGCAGCCCCCTGCTACTGAGAAGCTCCGGGATCCCAGCAGCCGCCACGCCCT
GGCCTCAGCCTGCGGGGCTCCAGTCAGGCCAACACCGACGCGCAGCTGGGAGGAAGACAGGACCCTTGACATCTCCATC

M   T   S   P   S   S   S   P
TGCACAGAGGTCTTGCTGGACCGAGCAGCCTCCTCCTTAGG ATG ACC TCA CCC TCC AGC TCT CCA      8
24

V   F   R   L   E   T   L   D   G   G   Q   E   D   G   S   E   A   D   R   G      28
GTT TTC AGG TTG GAG ACA TTA GAT GGA GGC CAA GAA GAT GGC TCT GAG GCG GAC AGA GGA      84

K   L   D   F   G   S   G   L   P   P   M   E   S   Q   F   Q   G   E   D   R      48
AAG CTG GAT TTT GGG AGC GGG CTG CCT CCC ATG GAG TCA CAG TTC CAG GGC GAG GAC CGG      144

K   F   A   P   Q   I   R   V   N   L   N   Y   R   K   G   T   G   A   S   Q      68
AAA TTC GCC CCT CAG ATA AGA GTC AAC CTC AAC TAC CGA AAG GGA ACA GGT GCC AGT CAG      204

P   D   P   N   R   F   D   R   D   R   L   F   N   A   V   S   R   G   V   P      88
CCG GAT CCA AAC CGA TTT GAC CGA GAT CGG CTC TTC AAT GCG GTC TCC CGG GST GTC CCC      264

E   D   L   A   G   L   P   E   Y   L   S   R   T   S   R   Y   L   T   D   S      108
GAG GAT CTG GCT GGA CTT CCA GAG TAC CTG AGC AAG ACC AGC AAG TAC CTC ACC GAC TCG      324

E   Y   T   E   G   S   T   G   K   T   C   L   M   K   A   V   L   N   L   K      128
GAA TAC ACA GAG GGC TCC ACA GGT AAG ACG TGC CTG ATG AAG GCT GTG CTG AAC CTT AAG      384

D   G   V   N   A   C   I   L   P   L   L   Q   I   D   R   D   S   G   N   P      148
GAC GGA GTC AAT GCC TGC ATT CTG CCA CTG CTG CAG ATC GAC AGG GAC TCT GGC AAT CCT      444

Q   P   L   V   N   A   Q   C   T   D   D   Y   Y   R   G   H   S   A   L   H      168
CAG CCC CTG GTA AAT GCC CAG TGC ACA GAT GAC TAT TAC CGA GGC CAC AGC GCT CTG CAC      504

I   A   I   E   K   R   S   L   Q   C   V   K   L   L   V   E   N   G   A   N      188
ATC GCC ATT GAG AAG AGG AGT CTG CAG TGT GTG AAG CTC CTG GTG GAG AAT GGG GCC AAT      564

V   H   A   R   A   C   G   R   F   F   Q   K   G   Q   G   T   C   F   Y   F      208
GTG CAT GCC CGG GCC TGC GGC CGC TTC TTC CAG AAG GGC CAA GGG ACT TGC TTT TAT TTC      624

G   E   L   P   L   S   L   A   A   C   T   K   Q   W   D   V   V   S   Y   L      228
GGT GAG CTA CCC CTC TCT TTG GCC GCT TGC ACC AAG CAG TGG GAT GTG GTA AGC TAC CTC      684

```

Fig. 2

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L	E	N	P	H	Q	P	A	S	L	Q	A	T	D	S	Q	G	N	T	V	248
CTG	GAG	AAC	CCA	CAC	CAG	CCC	GCC	AGC	CTG	CAG	GCC	ACT	GAC	TCC	CAG	GGC	AAC	ACA	GTC	744
L	H	A	L	V	M	I	S	D	N	S	A	E	N	I	A	L	V	T	S	268
CTG	CAT	GCC	CTA	GTG	ATG	ATC	TCG	GAC	AAC	TCA	GCT	GAG	AAC	ATT	GCA	CTG	GTG	ACC	ACC	804
M	Y	D	G	L	L	Q	A	G	A	R	L	C	P	T	V	Q	L	E	D	288
ATG	TAT	GAT	GGG	CTC	CTC	CAA	GCT	GGG	GCC	CGC	CTC	TGC	CCT	ACC	GTG	CAG	CTT	GAG	GAC	864
I	R	N	L	Q	D	L	T	P	L	K	L	A	A	K	E	G	K	I	E	308
ATC	GCC	AAC	CTG	CAG	GAT	CTC	ACG	CCT	CTG	AAG	CTG	GCC	GCC	AAG	GAG	GGC	AAG	ATC	GAG	924
I	F	R	H	I	L	Q	R	E	F	S	G	L	S	H	L	S	R	K	F	328
ATT	TTC	AGG	CAC	ATC	CTG	CAG	CGG	GAG	TTT	TCA	GGA	CTG	AGC	CAC	CTT	TCC	CGA	AAG	TTC	984
T	E	W	C	Y	G	P	V	R	V	S	L	Y	D	L	A	S	V	D	S	348
ACC	GAG	TGG	TGC	TAT	GGG	CCT	GTC	CGG	GTG	TCG	CTG	TAT	GAC	CTG	GCT	TCT	GTG	GAC	AGC	1044
C	E	E	N	S	V	L	E	I	I	A	F	H	C	K	S	P	H	R	H	368
TGT	GAG	GAG	AAC	TCA	GTG	CTG	GAG	ATC	ATT	GCC	TTT	CAT	TGC	AAG	AGC	CCG	CAC	CGA	CAC	1104
R	M	V	V	L	E	P	L	N	K	L	L	Q	A	K	W	D	L	L	I	388
CGA	ATG	GTC	GTT	TTG	GAG	CCC	CTG	AAC	AAA	CTG	CTG	CAG	GCG	AAA	TGG	GAT	CTG	CTC	ATC	1164
P	K	F	F	L	N	F	L	C	N	L	I	Y	M	F	I	F	T	A	V	408
CCC	AAG	TTC	TTC	TTA	AAC	TTC	CTG	TGT	AAT	CTG	ATC	TAC	ATG	TTC	ATC	TTC	ACC	GCT	GTT	1224
A	Y	H	Q	P	T	L	K	K	Q	A	A	P	H	L	K	A	E	V	G	428
GCC	TAC	CAT	CAG	CCT	ACC	CTG	AAG	AAG	CAG	GCC	GCC	CCT	CAC	CTG	AAA	GCG	GAG	GTT	GGA	1284
N	S	M	L	L	T	G	H	I	L	I	L	L	G	G	I	Y	L	L	V	448
AAC	TCC	ATG	CTG	CTG	ACG	GGC	CAC	ATC	CTT	ATC	CTG	CTA	GGG	GGG	ATC	TAC	CTC	CTC	GTG	1344
G	Q	L	W	Y	F	W	R	R	H	V	F	I	W	I	S	F	I	D	S	468
GGC	CAG	CTG	TGG	TAC	TTC	TGG	CGG	CGC	CAC	GTG	TTC	ATC	TGG	ATC	TCG	TTC	ATA	GAC	AGC	1404
Y	F	E	I	L	F	L	F	Q	A	L	L	T	V	V	S	Q	V	L	C	488
TAC	TTT	GAA	ATC	CTC	TTC	CTG	TTC	CAG	GCC	CTG	CTC	ACA	GTG	GTG	TCC	CAG	GTG	CTG	TGT	1464
F	L	A	I	E	W	Y	L	P	L	L	V	S	A	L	V	L	G	W	L	508
TTC	CTG	GCC	ATC	GAG	TGG	TAC	CTG	CCC	CTG	CTT	GTG	TCT	GCG	CTG	GTG	CTG	GGC	TGG	CTG	1524
N	L	L	Y	Y	T	R	G	F	Q	H	T	G	I	Y	S	V	M	I	Q	528
AAC	CTG	CTT	TAC	TAT	ACA	CGT	GGC	TTC	CAG	CAC	ACA	GGC	ATC	TAC	AGT	GTC	ATG	ATC	CAG	1584

Fig. 2 continued

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K V I L R D L L R F L L I Y L V F L F G 548
 AAG GTC ATC CTG CGG GAC CTG CTG CGC TTC CTT CTG ATC TAC TTA GTC TTC CTT TTC GGC 1644

 F A V A L V S L S Q E A W R P E A P T G 568
 TTC GCT GTA GCC CTG GTG AGC CTG AGC CAG GAG GCT TGG CGC CCC GAA GCT CCT ACA GGC 1704

 P N A T E S V Q P M E G Q E D E G N G A 588
 CCC AAT GCC ACA GAG TCA GTG CAG CCC ATG GAG GGA CAG GAG GAC GAG GGC AAC GGG GCC 1764

 Q Y R G I L E A S L E L F K F T I G M G 608
 CAG TAC AGG GGT ATC CTG GAA GCC TCC TTG GAG CTC TTC AAA TTC ACC ATC GGC ATG GGC 1824

 E L A F Q E Q L H F R G M V L L L L L A 628
 GAG CTG GCC TTC CAG GAG CAG CTG CAC TTC CGC GGC ATG GTG CTG CTG CTG CTG GGC 1884

 Y V L L T Y I L L L N M L I A L M S E T 648
 TAC GTG CTG CTC ACC TAC ATC CTG CTG CTC AAC ATG CTC ATC GCC CTC ATG AGC GAG ACC 1944

 V N S V A T D S W S I W K L Q K A I S V 668
 GTC AAC AGT GTC GCC ACT GAC AGC TGG AGC ATC TGG AAG CTG CAG AAA GCC ATC TCT GTC 2004

 L E M E N G Y W W C R K K Q R A G V M L 688
 CTG GAG ATG GAG AAT GGC TAT TGG TGG TGC AGG AAG AAG CAG CGG GCA GGT GTG ATG CTG 2064

 T V G T K P D G S P D E R W C F R V E E 708
 ACC GTT GGC ACT AAG CCA GAT GGC AGC CCG GAT GAG CGC TGG TGC TTC AGG GTG GAG GAG 2124

 V N W A S W E Q T L P T L C E D P S G A 728
 GTG AAC TGG GCT TCA TGG GAG CAG ACG CTG CCT ACG CTG TGT GAG GAC CCG TCA GGG GCA 2184

 G V P R T L E N P V L A S P P K E D E D 748
 GGT GTC CCT CGA ACT CTC GAG AAC CCT GTC CTG GCT TCC CCT CCC AAG GAG GAT GAG GAT 2244

 G A S E E N Y V P V Q L L Q S N * 765
 GGT GCC TCT GAG GAA AAC TAT GTG CCC GTC CAG CTC CTC CAG TCC AAC TGA 2295

TGGCCCAGATGCAGCAGGAGGCCAGAGGACAGAGCAGAGGATCTTTCCAACCACATCTGCTGGCTCTGGGGTCCCAGTG

AATTCTGGTGGCAAATATATATATTTTCACTAACTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Fig. 2 continued

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Partial human VR2 alternate form

Input file frhob12c4.seg; Output File frhob12c4.tra
Sequence length 1489

G	R	F	F	Q	K	G	Q	G	T	C	F	Y	F	G	E	L	P	L	19	
GC	GGC	CGC	TTC	TTC	CAG	AAG	GGC	CAA	GGG	ACT	TGC	TTT	TAT	TTC	GGT	GAG	CTA	CCC	CTC	57
S	L	A	A	C	T	K	Q	W	D	V	V	S	Y	L	L	E	N	P	H	39
TCT	TTG	GCC	GCT	TGC	ACC	AAG	CAG	TGG	GAT	GTG	GTA	AGC	TAC	CTC	CTG	GAG	AAC	CCA	CAC	117
Q	P	A	S	L	Q	A	T	D	S	Q	G	N	T	V	L	H	A	L	V	59
CAG	CCC	GCC	AGC	CTG	CAG	GCC	ACT	GAC	TCC	CAG	GGC	AAC	ACA	GTC	CTG	CAT	GCC	CTA	GTG	177
M	I	S	D	N	S	A	E	N	I	A	L	V	T	S	M	Y	D	G	L	79
ATG	ATC	TCG	GAC	AAC	TCA	GCT	GAG	AAC	ATT	GCA	CTG	GTG	ACC	AGC	ATG	TAT	GAT	GGG	CTC	237
L	Q	A	G	A	R	L	C	P	T	V	Q	L	E	D	I	R	N	L	Q	99
CTC	CAA	GCT	GGG	GCC	CGC	CTC	TGC	CCT	ACC	GTG	CAG	CTT	GAG	GAC	ATC	CGC	AAC	CTG	CAG	297
D	L	T	P	L	K	L	A	A	K	E	G	K	I	E	I	F	R	H	I	119
GAT	CTC	ACG	CCT	CTG	AAG	CTG	GCC	GCC	AAG	GAG	GGC	AAG	ATC	GAG	ATT	TTC	AGG	CAC	ATC	357
L	Q	R	E	F	S	G	L	S	H	L	S	R	K	F	T	E	W	C	Y	139
CTG	CAG	CGG	GAG	TTT	TCA	GGA	CTG	AGC	CAC	CTT	TCC	CGA	AAG	TTC	ACC	GAG	TGG	TGC	TAT	417
G	P	V	R	V	S	L	Y	D	L	A	S	V	D	S	C	E	E	N	S	159
GGG	CCT	GTC	CGG	GTG	TCG	CTG	TAT	GAC	CTG	GCT	TCT	GTG	GAC	AGC	TGT	GAG	GAG	AAC	TCA	477
V	L	E	I	I	A	F	H	C	K	S	P	H	R	H	R	M	V	V	L	179
GTG	CTG	GAG	ATC	ATT	GCC	TTT	CAT	TGC	AAG	AGC	CCG	CAC	CGA	CAC	CGA	ATG	GTC	GTT	TTG	537
E	P	L	N	K	L	L	Q	A	K	W	D	L	L	I	P	K	F	F	L	199
GAG	CCC	CTG	AAC	AAA	CTG	CTG	CAG	GCG	AAA	TGG	GAT	CTG	CTC	ATC	CCC	AAG	TTC	TTC	TTA	597
N	F	L	C	N	L	I	Y	M	F	I	F	T	A	V	A	Y	H	Q	P	219
AAC	TTC	CTG	TGT	AAT	CTG	ATC	TAC	ATG	TTC	ATC	TTC	ACC	GCT	GTT	GCC	TAC	CAT	CAG	CCT	657
T	L	K	K	Q	A	A	P	H	L	K	A	E	V	G	N	S	M	L	L	239
ACC	CTG	AAG	AAG	CAG	GCC	GCC	CCT	CAC	CTG	AAA	GCG	GAG	GTT	GGA	AAC	TCC	ATG	CTG	CTG	717
T	G	H	I	L	I	L	L	G	G	I	Y	L	L	V	G	Q	L	W	Y	259
ACG	GGC	CAC	ATC	CTT	ATC	CTG	CTA	GGG	GGG	ATC	TAC	CTC	CTC	GTG	GGC	CAG	CTG	TGG	TAC	777
F	W	R	R	H	V	F	I	W	I	S	F	I	D	S	Y	F	E	I	L	279
TTC	TGG	CGG	CGC	CAC	GTG	TTC	ATC	TGG	ATC	TCG	TTC	ATA	GAC	AGC	TAC	TTT	GAA	ATC	CTC	837

Fig. 3

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F	L	F	Q	A	L	L	T	V	V	S	Q	V	L	C	F	L	A	I	E	299
TTC	CTG	TTC	CAG	GCC	CTG	CTC	ACA	GTG	GTG	TCC	CAG	GTG	CTG	TGT	TTC	CTG	GCC	ATC	GAG	897
W	Y	L	P	L	L	V	S	A	L	V	L	G	W	L	N	L	L	Y	Y	319
TGG	TAC	CTG	CCC	CTG	CTT	GTG	TCT	GCG	CTG	GTG	CTG	GGC	TGG	CTG	AAC	CTG	CTT	TAC	TAT	957
T	R	G	F	Q	H	T	G	I	Y	S	V	M	I	Q	K	K	A	I	S	339
ACA	CGT	GGC	TTC	CAG	CAC	ACA	GGC	ATC	TAC	AGT	GTC	ATG	ATC	CAG	AAG	AAA	GCC	ATC	TCT	1017
V	L	E	M	E	N	G	Y	W	W	C	R	K	K	Q	R	A	G	V	M	359
GTC	CTG	GAG	ATG	GAG	AAT	GGC	TAT	TGG	TGG	TGC	AGG	AAG	AAG	CAG	CGG	GCA	GGT	GTG	ATG	1077
L	T	V	G	T	K	P	D	G	S	P	D	E	R	W	C	F	R	V	E	379
CTG	ACC	GTT	GGC	ACT	AAG	CCA	GAT	GGC	AGC	CCG	GAT	GAG	CGC	TGG	TGC	TTC	AGG	GTG	GAG	1137
E	V	N	W	A	S	W	E	Q	T	L	P	T	L	C	E	D	P	S	G	399
GAG	GTG	AAC	TGG	GCT	TCA	TGG	GAG	CAG	ACG	CTG	CCT	ACG	CTG	TGT	GAG	GAC	CCG	TCA	GGG	1197
A	G	V	P	R	T	L	E	N	P	V	L	A	S	P	P	K	E	D	E	419
GCA	GGT	GTC	CCT	CGA	ACT	CTC	GAG	AAC	CCT	GTC	CTG	GCT	TCC	CCT	CCC	AAG	GAG	GAT	GAG	1257
D	G	A	S	E	E	N	Y	V	P	V	Q	L	L	Q	S	N	*			437
GAT	GGT	GCC	TCT	GAG	GAA	AAC	TAT	GTG	CCC	GTC	CAG	CTC	CTC	CAG	TCC	AAC	TGA			1311

TGGCCCAGATGCAGCAGGAGGCCAGAGGACAGAGCAGAGGATCTTTCCAACCACATCTGCTGGCTCTGGGGTCCCAGTG

AATTCTGGTGGCAAATATATATTTTCACTAACTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGA

GCGGACGCGTGGGTCGAC

Fig. 3 continued

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Partial rat VR2

Input file Flrxb147g11.seq; Output File Flrxb147g11.tra

Sequence length 1794

S	T	H	A	S	A	L	S	L	A	A	C	T	K	Q	W	D	V	V		19
G	TCG	ACC	CAC	GCG	TCC	GCT	CTT	TCT	CTG	GCT	GCG	TGC	ACC	AAG	CAG	TGG	GAT	GTG	GTG	57
T	Y	L	L	E	N	P	H	Q	P	A	S	L	E	A	T	D	S	L	G	39
ACC	TAC	CTC	CTG	GAG	AAC	CCA	CAC	CAG	CCG	GCC	AGC	CTG	GAG	GCC	ACC	GAC	TCC	CTG	GGC	117
N	T	V	L	H	A	L	V	M	I	A	D	N	S	P	E	N	S	A	L	59
AAC	ACA	GTC	CTG	CAT	GCT	CTG	GTA	ATG	ATT	GCA	GAT	AAC	TCG	CCT	GAG	AAC	AGT	GCC	CTG	177
V	I	H	M	Y	D	G	L	L	Q	M	G	A	R	L	C	P	T	V	Q	79
GTG	ATC	CAC	ATG	TAC	GAC	GGG	CTT	CTA	CAA	ATG	GGG	GCG	CGC	CTC	TGC	CCC	ACT	GTG	CAG	237
L	E	E	I	S	N	H	Q	G	L	T	P	L	K	L	A	A	K	E	G	99
CTT	GAG	GAA	ATC	TCC	AAC	CAC	CAA	GGC	CTC	ACA	CCC	CTG	AAA	CTA	GCC	GCC	AAG	GAA	GGC	297
K	I	E	I	F	R	H	I	L	Q	R	E	F	S	G	P	Y	Q	P	L	119
AAA	ATC	GAG	ATT	TTC	AGG	CAC	ATT	CTG	CAG	CGG	GAA	TTC	TCA	GGA	CCG	TAC	CAG	CCC	CTT	357
S	R	K	F	T	E	W	C	Y	G	P	V	R	V	S	L	Y	D	L	S	139
TCC	CGA	AAG	TTT	ACT	GAG	TGG	TGT	TAC	GGT	CCT	GTG	CGG	GTA	TCG	CTG	TAC	GAC	CTG	TCC	417
S	V	D	S	W	E	K	N	S	V	L	E	I	I	A	F	H	C	K	S	159
TCT	GTG	GAC	AGC	TGG	GAA	AAG	AAC	TCG	GTG	CTG	GAG	ATC	ATC	GCT	TTT	CAT	TGC	AAG	AGC	477
P	N	R	H	R	M	V	V	L	E	P	L	N	K	L	L	Q	E	K	W	179
CCG	AAC	CGG	CAC	CGC	ATG	GTG	GTT	TTA	GAA	CCA	CTG	AAC	AAG	CTT	CTG	CAG	GAG	AAA	TGG	537
D	R	L	V	S	R	P	F	F	N	F	A	C	Y	L	V	Y	M	F	I	199
GAT	CGG	CTC	GTC	TCA	AGA	TTC	TTC	TTC	AAC	TTC	GCC	TGC	TAC	TTG	GTC	TAC	ATG	TTC	ATC	597
F	T	V	V	A	Y	H	Q	P	S	L	D	Q	P	A	I	P	S	S	K	219
TTC	ACC	GTC	GTT	GCC	TAC	CAC	CAG	CCT	TCC	CTG	GAT	CAG	CCA	GCC	ATC	CCC	TCA	TCA	AAA	657
A	T	F	G	E	S	M	L	L	L	G	H	I	L	I	L	L	G	G	I	239
GCG	ACT	TTT	GGG	GAA	TCC	ATG	CTG	CTG	CTG	GGC	CAC	ATT	CTG	ATC	CTG	CTT	GGG	GGT	ATT	717
Y	L	L	L	G	Q	L	W	Y	F	W	R	R	R	L	F	I	W	I	S	259
TAC	CTC	TTA	CTG	GGC	CAG	CTG	TGG	TAC	TTT	TGG	CGG	CGG	CGC	CTG	TTT	ATC	TGG	ATC	TCA	777
F	M	D	S	Y	F	E	I	L	F	L	L	Q	A	L	L	T	V	L	S	279
TTC	ATG	GAC	AGC	TAC	TTT	GAA	ATC	CTC	TTT	CTC	CTT	CAG	GCT	CTG	CTC	ACA	GTG	CTG	TCC	837

Fig. 4

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Q	V	L	R	F	M	E	T	E	W	Y	L	P	L	L	V	L	S	L	V	299
CAG	GTG	CTG	CGC	TTC	ATG	GAG	ACT	GAA	TGG	TAC	CTA	CCC	CTG	CTA	GTG	TTA	TCC	CTA	GTG	897
L	G	W	L	N	L	L	Y	Y	T	R	G	F	Q	H	T	G	I	Y	S	319
CTG	GGC	TGG	CTG	AAC	CTG	CTT	TAC	TAC	ACA	CGG	GGC	TTT	CAG	CAC	ACA	GGC	ATC	TAC	AGT	957
V	M	I	Q	K	V	I	L	R	D	L	L	R	F	L	L	V	Y	L	V	339
GTC	ATG	ATC	CAG	AAG	GTC	ATC	CTT	CGA	GAC	CTG	CTC	CGT	TTC	CTG	CTG	GTC	TAC	CTG	GTC	1017
F	L	F	G	F	A	V	A	L	V	S	L	S	R	E	A	R	S	P	K	359
TTC	CTT	TTC	GGC	TTT	GCT	GTA	GCC	CTA	GTA	AGC	TTG	AGC	AGA	GAG	GCC	CGA	AGT	CCC	AAA	1077
A	P	E	D	N	N	S	T	V	T	E	Q	P	T	V	G	Q	E	E	E	379
GCC	CCT	GAA	GAT	AAC	AAC	TCC	ACA	GTG	ACG	GAA	CAG	CCC	ACG	GTG	GGC	CAG	GAG	GAG	GAG	1137
P	A	P	Y	R	S	I	L	D	A	S	L	E	L	F	K	F	T	I	G	399
CCA	GCT	CCA	TAT	CGG	AGC	ATT	CTG	GAT	GCC	TCC	CTA	GAG	CTG	TTC	AAG	TTC	ACC	ATT	GGT	1197
M	G	E	L	A	F	Q	E	Q	L	R	F	R	G	V	V	L	L	L	L	419
ATG	GGG	GAG	CTG	GCT	TTC	CAG	GAA	CAG	CTG	CGT	TTT	CGT	GGG	GTG	GTC	CTG	CTG	TTG	CTG	1257
L	A	Y	V	L	L	T	Y	V	L	L	L	N	M	L	I	A	L	M	S	439
TTG	GCC	TAC	GTC	CTT	CTC	ACC	TAC	GTC	CTG	CTG	CTC	AAC	ATG	CTC	ATT	GCT	CTC	ATG	AGC	1317
E	T	V	N	H	V	A	D	N	S	W	S	I	W	K	L	Q	K	A	I	459
GAA	ACT	GTC	AAC	CAC	GTT	GCT	GAC	AAC	AGC	TGG	AGC	ATC	TGG	AAG	TTG	CAG	AAA	GCC	ATC	1377
S	V	L	E	M	E	N	G	Y	W	W	C	R	R	K	K	H	R	E	G	479
TCT	GTC	TTG	GAG	ATG	GAG	AAT	GGT	TAC	TGG	TGG	TGC	CGG	AGG	AAG	AAA	CAT	CGT	GAA	GGG	1437
R	L	L	K	V	G	T	R	G	D	G	T	P	D	E	R	W	C	F	R	499
AGG	CTG	CTG	AAA	GTC	GGC	ACC	AGG	GGG	GAT	GGT	ACC	CCT	GAT	GAG	CGC	TGG	TGC	TTC	AGG	1497
V	E	E	V	N	W	A	A	W	E	K	T	L	P	T	L	S	E	D	P	519
GTG	GAG	GAA	GTA	AAT	TGG	GCT	GCT	TGG	GAG	AAG	ACT	CTT	CCC	ACC	TTA	TCT	GAG	GAT	CCA	1557
S	G	P	G	I	T	G	N	K	K	N	P	T	S	K	P	G	K	N	S	539
TCA	GGG	CCA	GGC	ATC	ACT	GGT	AAT	AAA	AAG	AAC	CCA	ACC	TCT	AAA	CCG	GGG	AAG	AAC	AGT	1617
A	S	E	E	D	H	L	P	L	Q	V	L	Q	S	P	*					555
GCC	TCA	GAG	GAA	GAC	CAT	CTG	CCC	CTT	CAG	GTC	CTC	CAG	TCC	CCC	TGA					1665

TGGCCCAGATGCAGCAGCAGGCTGGCAGGATGGAGTAGGGAATCTTCCCAGCCACACCAGAGGCTACTGAATTTTGGTG

GAAATATAAATATTTTTTTTGCATAAAAAAAAAAAAAAAGGGCGGCCGC

Fig. 4 continued

```

1 .....MTSPSSSPVF 10
1 MKKWSSTD LGTAADPLQKDTCPDPLDGPNSRPPPAKPQLPTAKSRTLRF 50
11 RLETL DGGQEDGSEADRGKLD FGSGLPPMESQFQGEDRK FAPQIRVNLNY 60
51 GKGDSEEA FVDCPHEEGEL DSCPTI.TVSPVITIQRPGDGPTGARLLSQ 99
61 RKG TGASQPD PNRFDRLFN AVSRGVPEDLAGLPEYLSKTSKYLTDSEY 110
100 DSVAASTEKTLRLYDRRSIFEAVAQNNCQDLESLLLFLQSKKHLTDNEF 149
111 TEGSTGKTCLMKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNAQCTDDY 160
150 KDPETGKTCLLKAMLN LHDGQNTTIPLLEIARQ TDSLKELVNASYTDSY 199
161 YRGHSALHIAIEKRS LQCVKLLVENGANV HARACGRFFQKGQG.TCFYFG 209
200 YKGOTALHIAIERRN MALVTLLVENGADVOAAAHGDFFKTKGRPGFYFG 249

```

Fig. 5

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210 ELPLSLAACTKQWDVVSYLENPHQPASLQATDSQGNTVLHALVMISDNS 259
   ||||| | : | : || : | | : | | ||||| : . || .
250 ELPLSLAACTNQLGIVKFLQLNSWQTADISARDSVGNTVLHALVEVADNT 299
   .
260 AENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKLAKEGKIEI 309
   | : | | | | . : | | : | | . . || : : | . : || | | | | :
300 ADNTKFVTSMYNEILMLGAKLHPTLKLEELTNKKGMTPLALAAGTGKIGV 349
   .
310 FRHILQREFS..GLSHLSRKFTWCYGPVVRVSLYDLASVDSCEENSVLEI 357
   : || | | | | | | | | | | | | | | | | | | . : | . | | | | :
350 LAYILQREIQEPECRHLSRKFTWAYGPHSSLYDLSCIDTCEKNSVLEV 399
   .
358 IAF.HCKSPHRHRMVVLEPLNKLQAKWDLIPK.FFLNFLCNLIYMFIF 405
   || : . . | . | | | . . . | | : | | | | | : : | : | | | : | | |
400 IAYSSSETPNRHDMLLVEPLNRLQDKWDRFVKRIFYFNFLVYCLYMIIF 449
   .
406 TAVAYHQPTLKKQAAPHLKAEVGNMMLLTGHILILLGGIYLLVGQLWYFW 455
   | | : . | | | | | : . : | | | | | . | | : | | : | |
450 TMAAYYRPV..DGLPPFKMEKIGDYFRVTGEILSVLGGVYFFFRGIQYFL 497
   .
456 RRHVFIWISFIDSYFEILFLFQALLTVVSQVLCFLAIEWYLPLLVSALVL 505
   . | . . : | | | | | . | | | | . . | | | | . : | . | |
498 QRRPSMKTFLVDSYSEMLFFLQSLFMLATVVLYFSHLKEYVASMVFSAL 547
   .
506 GWLNLLYYTRGFQHTGIYSVMIQKVILRDLRLFLLIYLVFLFGFAVALVS 555
   || : | | | | | | | | | | : | . | | | | | : : | : | | | | . | . |
548 GWTNMLYYTRGFQQMGIYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVT 597
   .
556 LSQEAWRPEAPTGPNATESVQPMEGQDEGNGAQYRGILEASLELFKFTI 605
   | : : | . . | | | | | | | | | | | | : | | | | |
598 LIEDGKNDSLPSSESTSHRWGPACRPPD....SSYNSLYSTCLELFKFTI 643
   .
606 GMGELAFQEQLHFRGMVLLLLLAYVLLTYILLNMLIALMSETVNSVATD 655
   || : | | | | : . : | | | | | : | | | | | | | | | | | | : | :
644 GMGDLEFTENYDFKAVFIILLLAYVILTYILLNMLIALMGETVNKIAQE 693
   .
656 SWSIWKLQKAISVLEMENGYWWC.RKKQRAQVMLTVGTPDGSPDERWCF 704
   | . | | | | : | | : | : | : | | | | | | | | | | | |
694 SKNIWKLQRAITILDTEKSFLKCMRKAFRSGKLLQVGYTPDGKDDYRWCF 743
   .
705 RVEEVNWSWEQTLPTLCEDPSGA.GVPRTLENPVLASPPKEDEDGASEE 753
   || : | | | | . | . : | | | | | | | | | | . | |
744 RVDEVNWTTWNTNVGIINEDPGNCEGVKRTLSFSLRSS....RVSGRHWK 789
   .
754 NYVPVQLLQSN..... 764
   | : | | | .
790 NFALVPLLREASARDRQSAQPEEVYLRQFSGSLKPEDAEVFKSPAASGEK 839

```

Fig. 5 continued

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GAP of: humanvr2.seq check: 8853 from: 1 to: 2809

humanVR2 21e11a, 2809 bases, 8853 checksum.

to: humanvr1.seq check: 4554 from: 1 to: 3909

humanVR1 Fbh18547pat - Import - complete

Symbol comparison table:

/ddm_local/gcg/gcg_9.1/gcgcore/data/rundata/nwsgapdna.cmp

CompCheck: 8760

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	0.000

Quality:	14359	Length:	3934
Ratio:	5.112	Gaps:	15
Percent Similarity:	55.316	Percent Identity:	55.316

Match display thresholds for the alignment(s):

	=	IDENTITY
:	=	5
.	=	1

humanvr2.seq x humanvr1.seq

```

      .
      .
      .
1 .....GGCTAGCCTGTCCTGACAGGGGAGAG 26
      | | | | | | | |
801 TGTCCACAGTAGTCCCCCTTATCCACGGGTGTCACTTTCCATGGGTTCA 850
      .
      .
27 TTAAGCTCCCGTTCTCCACCGTGCCGGCTGGCCAGGTGGGCTGAGGGTGA 76
      | | | | | | | | | |
851 GTTATTTGCGGTCAACCACGGTCTGCCAATATTAAATGGAAAATTCTTCA 900
      .
      .
77 CCGAGAGACCAGAACCTGCTTGCTGGAGCTTAGTGCTCAGAGCTGGGGAG 126
      | | | | | | | | | |
901 AACAGTTCCCAAGTTTCCCTTGTGCATTGTTCTGAGCAGTGTGATGAAG 950
      .
      .
127 GGAGGTCCGCGCTCCTCTGCTGTCAGCGCCGGCAGCCCTCCCGGCTT 176
      | | | | | | | | | |
951 AGTCTCTGCCGTGCCATCTGGGATGCAAACCGTCCCTGTGTCCCCCACGT 1000

```

Fig. 6

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177 CACTTCCTCCCGCAGCCCCTGCTACTGAGAAGCTCCGGGATCCCAGCAGC 226
| | | | | | | | | | | | | | | | | | | | | |
1001 CCAGGCCGTAGATGCTCCCCGCCGGTCAGTCACTTAGTCGTCAGATCGCC 1050
227 CGCCACGCCCTGGC.....CTCAGCCTGCGGG 253
| | | | | | | | | | | | | | | | | | | | | |
1051 CGTCCTGGTATCACAGTGCTTCTGTTCAGGTTGCACACTGGGCCACAGAG 1100
254 GCTCCAGTCAGGCCAACACCGACGCGCAGCTGGGAGGAAG..... 293
| | | | | | | | | | | | | | | | | | | | | |
1101 GATCCAGCAAGGATGAAGAAATGGAGCAGCACAGACTTGGGGACAGCTGC 1150
294ACAGGACCCTTGACATCTCCATCTGCACAGAGGTCTCTG 331
| | | | | | | | | | | | | | | | | | | | | |
1151 GGACCCACTCCAAAAGGACACCTGCCCAGACCCCCTGGATGGAGACCCTA 1200
332 GCTGGACCGAGCAGCCTCCTCCTCCTAGGATGACCTCACCTCCAGC..T 379
| | | | | | | | | | | | | | | | | | | | | |
1201 ACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCCCCACGGCCAAGAGCCGC 1250
380 CTCCAGTTTTCAGGTTGGAGACATTAGATGGAGGCCAAGAAGATGGCTCT 429
| | | | | | | | | | | | | | | | | | | | | |
1251 ACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTCCCGGTGGATTG 1300
430 GAGGCGGACAGAGGAAAGCTGGATTTTGGGAGCGGGCTGCCTCCCATGGA 479
| | | | | | | | | | | | | | | | | | | | | |
1301 CCCCCACGAGGAAGGTGAGTTGGACTCCTGCCCCGACCATCACAGTCAGCC 1350
480 GTCACAGTTCCAGGGCGAGGACCGGAAATTCGCCCCTCAGATAAGAGTCA 529
| | | | | | | | | | | | | | | | | | | | | |
1351 CTGTTATCACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGG..C 1398
530 ACCTCAACTACCGAAAGGGAACAGGTGCCAGTCAGCCGGATCCAAACCGA 579
| | | | | | | | | | | | | | | | | | | | | |
1399 TGCTGTCCCAGGACTCTGTGCGCCGACGACCGAGAAGACCCTCAGGCTC 1448
580 TTTGACCGAGATCGGCTCTTCAATGCGGTCTCCCGGGGTGTCCCCGAGGA 629
| | | | | | | | | | | | | | | | | | | | | |
1449 TATGATCGCAGGAGTATCTTTGAAGCCGTTGCTCAGAATAACTGCCAGGA 1498
630 TCTGGCTGGACTTCCAGAGTACCTGAGCAAGACCAGCAAGTACCTCACCG 679
| | | | | | | | | | | | | | | | | | | | | |
1499 TCTGGAGAGCCTGCTGCTCTTCTGTCAGCAAGAGCAAGAAGCACCTCACAG 1548

Fig. 6 continued

680 ACTCGGAATACACAGAGGGCTCCACAGGTAAGACGTGCCTGATGAAGGCT 729
||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1549 ACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC 1598

730 GTGCTGAACCTTAAGGACGGAGTCAATGCCTGCATTCTGCCACTGCTGCA 779
||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1599 ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGA 1648

780 GATCGACAGGGACTCTGGCAATCCTCAGCCCCTGGTAAATGCCCAGTGCA 829
||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1649 GATCGCGCGGCAAACGGACAGCCTGAAGGAGCTTGTC AACGCCAGCTACA 1698

830 CAGATGACTATTACCGAGGCCACAGCGCTCTGCACATCGCCATTGAGAAG 879
| | | ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1699 CGGACAGCTACTACAAGGGCCAGACAGCACTGCACATCGCCATCGAGAGA 1748

880 AGGAGTCTGCAGTGTGTGAAGCTCCTGGTGGAGAATGGGGCCAATGTGCA 929
| | ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1749 CGCAACATGGCCCTGGTGACCCTCCTGGTGGAGAACGGAGCAGACGTCCA 1798

930 TGCCCGGGCCTGCGGCCGCTTCTTCCAGAAGGGCCAAG...GGACTTGCT 976
||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1799 GGCTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGGCGGCCTGGAT 1848

977 TTTATTTTCGGTGAGCTACCCCTCTCTTTGGCCGCTTGACCAAGCAGTGG 1026
| | | ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1849 TCTACTTCGGTGAACTGCCCTGTCCCTGGCCGCGTGACCAACCAGCTG 1898

1027 GATGTGGTAAGCTACCTCCTGGAGAACCCACACCAGCCCGCCAGCCTGCA 1076
| | | ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1899 GGCATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAG 1948

1077 GGCCACTGACTCCCAGGGCAACACAGTCCTGCATGCCCTAGTGATGATCT 1126
||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1949 CGCCAGGGACTCGGTGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGG 1998

1127 CGGACAACTCAGCTGAGAACATTGCACTGGTGACCAGCATGTATGATGGG 1176
| | ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

1999 CCGACAACACGGCCGACAACACGAAGTTTGTGACGAGCATGTACAATGAG 2048

1177 CTCCTCCAAGCTGGGGCCCGCCTCTGCCCTACCGTGCAGCTTGAGGACAT 1226
| | | ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

2049 ATTCTGATGCTGGGGGCCAAACTGCACCCGACGCTGAAGCTGGAGGAGCT 2098

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1227 CCGCAACCTGCAGGATCTCACGCCTCTGAAGCTGGCCGCCAAGGAGGGCA 1276
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2099 CACCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCTGGGACCGGGA 2148
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1277 AGATCGAGATTTTCAGGCACATCCTGCAGCGGGAGTT.....TTCAGGA 1320
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2149 AGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAG 2198
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1321 CTGAGCCACCTTTCCCGAAAGTTCACCGAGTGGTGCTATGGGCCTGTCCG 1370
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2199 TGCAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCCGTGCA 2248
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1371 GGTGTCGCTGTATGACCTGGCTTCTGTGGACAGCTGTGAGGAGAACTCAG 1420
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2249 CTCCTCGCTGTACGACCTGTCCTGCATCGACACCTGCGAGAAGAAGTCCG 2298
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1421 TGCTGGAGATCATTGCCTTTCATTGCA...AGAGCCCGCACCGACACCGA 1467
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2299 TGCTGGAGGTGATCGCCTACAGCAGCAGCGAGACCCCTAATCGCCACGAC 2348
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1468 ATGGTCGTTTTGGAGCCCCCTGAACAAACTGCTGCAGGCGAAATGGGA... 1514
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2349 ATGCTCTTGGTGGAGCCGCTGAACCGACTCCTGCAGGACAAGTGGGACAG 2398
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1515 TCTGCTCATCCCCAAGTTCTTCTTAAACTTCCTGTGTAATCTGATCTACA 1564
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2399 ATTCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTACTGCCTGTACA 2448
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1565 TGTTTCATCTTCACCGCTGTTGCCTACCATCAGCCTACCCCTGAAGAAGCAG 1614
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2449 TGATCATCTTCACCATGGCTGCCTACTA....CAGGCCCGTGGATGGCTT 2494
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1615 GCCGCCCTCACCTGAAAGCGGAGGTTGGAAACTCCATGCTGCTGACGGG 1664
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2495 GCCTCCCTTTA..AGATGGAAAAAATTGGAGACTATTTCCGAGTTACTGG 2542
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1665 CCACATCCTTATCCTGCTAGGGGGGATCTACCTCCTCGTGGGCCAGCTGT 1714
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2543 AGAGATCCTGTCTGTGTTAGGAGGAGTCTACTTCTTTTTCCGAGGGATTC 2592
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
1715 GGTACTTCTGGCGGCCACGTGTTTCATCTGGATCTCGTTCATAGACAGC 1764
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
2593 AGTATTTCTGCAGAGGCGGCCGTCGATGAAGACCCTGTTTGTGGACAGC 2642
| | | | | | | | | | | | | | | | | | | | | | | | | | | |

Fig. 6 continued

1765 TACTTTGAAATCCTCTTTCCTGTTCAGGCCCTGCTCACAGTGGTGTCCCA 1814
||| ||| || | | | | | | | | | | | | | | |
2643 TACAGTGAGATGCTTTTCTTTCTGCAGTCACTGTTTCATGCTGGCCACCGT 2692

1815 GGTGCTGTGTTTTCTGGCCATCGAGTGGTACCTGCCCCCTGCTTGTGTCTG 1864
||||||| ||| | | | | | | | | | | | | | | |
2693 GGTGCTGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTATTCT 2742

1865 CGCTGGTGCTGGGCTGGCTGAACCTGCTTTACTATAACAGTGGCTTCCAG 1914
| | | | | | | | | | | | | | | | | | | | | | | | | | |
2743 CCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAG 2792

1915 CACACAGGCATCTACAGTGTTCATGATCCAGAAGGTCATCCTGCGGGACCT 1964
|| | | | | | | | | | | | | | | | | | | | | | | | | | |
2793 CAGATGGGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCT 2842

1965 GCTGCGCTTCCTTCTGATCTACTTAGTCTTCCTTTTCGGCTTCGCTGTAG 2014
| | | | | | | | | | | | | | | | | | | | | | | | | | |
2843 GTGCCGTTTCATGTTTGTCTACATCGTCTTCTTGTTTCGGGTTTTCCACAG 2892

2015 CCCTGGTGAGCCTGAGCCAGGAGGCTTGGCGCCCCGAAGCTCCTACAGGC 2064
| | | | | | | | | | | | | | | | | | | | | | | | | | |
2893 CGGTGGTGACGCTGATTGAAGACGGGAAGAATGACTCCCTGCCGTCTGAG 2942

2065 CCCAATGCCACAGAGTCAGTGCAGCCCATGGAGGGACAGGAGGACGAGGG 2114
||| | | | | | | | | | | | | | | | | | | | | | | | | |
2943 TCCA.....CGTCGCACAGGTGGCGGGGGCCTGCCTGCAGGCC 2980

2115 CAACGGGGCCCAGTACAGGGGTATCCTGGAAGCCTCCTTGGAGCTCTTCA 2164
| | | | | | | | | | | | | | | | | | | | | | | | | | |
2981 CCCCgATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGTTCA 3030

2165 AATTcACCATCGGCATGGGCGAGCTGGCCTTCCAGGAGCAGCTGCATTc 2214
| | | | | | | | | | | | | | | | | | | | | | | | | | |
3031 AGTTcACCATCGGCATGGGCGACCTGGAGTTCACTGAGAACTATGACTTc 3080

2215 CGCGGCATGGTGTCTGCTGCTGCTGCTGGCCTACGTGCTGCTCACCTACAT 2264
| | | | | | | | | | | | | | | | | | | | | | | | | | |
3081 AAGGCTGTCTTCATCATCCTGCTGCTGGCCTATGTAATTCTCACCTACAT 3130

2265 CCTGCTGCTCAACATGCTCATCGCCCTCATGAGCGAGACCGTCAACAGTG 2314
||| | | | | | | | | | | | | | | | | | | | | | | | | |
3131 CCTCCTGCTCAACATGCTCATCGCCCTCATGGGTGAGACTGTCAACAAGA 3180

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2315 TCGCCACTGACAGCTGGAGCATCTGGAAGCTGCAGARAGCCATCTCTGTGTC 2364
||| | | | | | | | | | | | | | | | | | | | | | | | | |
3181 TCGCACAGGAGAGCAAGAACATCTGGAAGCTGCAGAGAGCCATCACCATC 3230

2365 CTGGAGATGGAGAATGGCTATTGGTGGTGCAGGAAGAAG...CAGCGGGC 2411
||| | | | | | | | | | | | | | | | | | | | | | | | |
3231 CTGGACACGGAGAAGAGCTTCCTTAAGTGCATGAGGAAGGCCCTTCCGCTC 3280

2412 AGGTGTGATGCTGACCGTTGGCACTAAGCCAGATGGCAGCCCGGATGAGC 2461
||| | | | | | | | | | | | | | | | | | | | | | | |
3281 AGGCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACTACC 3330

2462 GCTGGTGCTTCAGGGTGGAGGAGGTGAACTGGGCTTCATGGGAGCAGACG 2511
| | | | | | | | | | | | | | | | | | | | | | | | | |
3331 GGTGGTGCTTCAGGGTGGACGAGGTGAACTGGACCACCTGGAACACCAAC 3380

2512 CTGCCTACGCTGTGTGAGGACCCG...TCAGGGGCAGGTGTCCCTCGAAC 2558
| | | | | | | | | | | | | | | | | | | | | | | | | |
3381 GTGGGCATCATCAACGAAGACCCGGGCAACTGTGAGGGCGTCAAGCGCAC 3430

2559 TCTCGAGAACCTGTCTG...GCTTCCCCTCCAAGGAGGATGAGGAT 2604
| | | | | | | | | | | | | | | | | | | | | | | | | |
3431 CCTGAGCTTCTCCCTGCGGTCAAGCAGAGTTTCAGGCAGACACTGGAAGA 3480

2605 GGTGCCTCTGAGGAAAACATGTGCCCCGTCCAGCTCCTCCAGTCCAACTG 2654
| | | | | | | | | | | | | | | | | | | | | | | | | |
3481 ACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCTCGAGATAGGCAG 3530

2655 ATGGCCCAGATGCAGCAGGAGGCCAGAGGACAGAGCAGAGGATCTTTCCA 2704
| | | | | | | | | | | | | | | | | | | | | | | | | |
3531 TCTGCTCAGCCCAGGAAGTTTATCTGCGACAGTTTTTCAGGGTCTCTGAA 3580

2705 ACCACATCTGCTGGCTCTGGGGTCCCAGTGAATTCTGGTGGCAAATATAT 2754
| | | | | | | | | | | | | | | | | | | | | | | | | |
3581 GCCA.....GAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGGGGA 3625

2755 ATTTTCACTAACTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2804
| | | | | | | | | | | | | | | | | | | | | | | | | |
3626 GAAGTGAGGACGTCACGCAGACAGCACTGTCAACACTGGGCCTTAGGAGA 3675

2805 AAAAA..... 2809

3676 CCCCGTTGCCACGGGGGGCTGCTGAGGGAACACCAGTGCTCTGTCAGCAG 3725

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CLUSTAL W (1.74) multiple sequence alignment

```

humanVR2      MTSPSSSPVFRLETLDGGQEDGSEADRGKLDGSGLPPEMESQFQGEDRKFPQIRVNLNY
rat VR2      -----

humanVR2      RKG TGASQPD PNRFD RDR LFN AVSRGVPEDLAGLPEYLSKTSKYLT DSEYTEGSTGKTCL
rat VR2      -----

humanVR2      MKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLQCVK
rat VR2      -----

humanVR2      LLVENGANVHARACGRFFQKGQGTCTFYFGELPLSLAACTKQWDVVSYLLENPHQPASLQA
rat VR2      -----STHASALSLAACTKQWDVVTYLLENPHQPASLEA
                      .*****:*****:
                      .*****:*****:

humanVR2      TDSQGNTVLHALVMISDNSAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKL
rat VR2      TDSLGN TVLHALVM IADNSPENSALVIHMYDGLLQMGARLCPTVQLEEI SNHQLTPLKL
                      *** *****:***.*** ***** *****:.* * .*****

humanVR2      AAKEGKIEIFRHILQREFSG-LSHLSRKFTWCYGPVVRVSLYDLASVDSCEENS VLEIIA
rat VR2      AAKEGKIEIFRHILQREFSGPYQPLSRKFTWCYGPVVRVSLYDLSSVDSWEKNSVLEIIA
                      ***** . *****:**** *:*****

humanVR2      FHCKSPHRHRMVVLEPLNKLLOAKWDL LIPKFFLNFLCNLIYMFIFTAVAYHQPTLKKQA
rat VR2      FHCKSPNRHRMVVLEPLNKLLOEKWDR LVS RFFNFACYLVMFIFTV VAYHQPSLDQPA
                      *****:***** *** *:.*:*** * *:*****.*****:.*: *

humanVR2      APHLKAEVGNSMLLTGHILILLGGIYLLVGQLWYFWRRHVFIWISFIDSYFEILFLFQAL
rat VR2      IPSSKATFGESMLLLGHILILLGGIYLLVGQLWYFWRRRLFIWISFMDSYFEILFLLQAL
                      * ** .*:**** *****:*****:*****:*****:***

humanVR2      LTVVSQVLCFLAIEWYLP LLVSALVLGWLNL LYYTRGFQHIGIYSVMIQKVILRDLLRFL
rat VR2      LTVLSQVLRFMETEWYLP LLVLSVLGWLNL LYYTRGFQHTGIYSVMIQKVILRDLLRFL
                      ***:**** *: ***** .*****:*****:*****:*****:*****

humanVR2      LIYLVFLFGFAVALVSLSQEAWRPEAPTGNATESVQPMEGQEDGNGAQYRGILEASLE
rat VR2      LVYLVFLFGFAVALVSLSREARSPKAPEDNNSTVTEQPTVGQEEEP--APYRSILDASLE
                      *:*****:*** *:.* .*: * ** *:***: * **.*:****

humanVR2      LFKFTIGMGELAFQEQLHFRGMVLLLLL LAYVLLTYILLNMLIALMSETVNSVATDSWSI
rat VR2      LFKFTIGMGELAFQEQLRFRGVLLLLL LAYVLLTYVLLNMLIALMSETVNHVADNSWSI
                      *****:***:*****:*****:*****:***** ** :****

humanVR2      WKLQKAISVLEMENGYWWCR-KKQ RAGVMLTVGTPDGSPDERWC FRVEEVN WASWEQTL
rat VR2      WKLQKAISVLEMENGYWWCRRKKHREGRL LKVGTRGDGTPDERWC FRVEEVN KAWEKTL
                      ***** **.* *:.*: **:*:*****:***:

humanVR2      PTLCEDPSGAGVPRTLENPVLASPPKEDEDGASEENYVPVQLLQSN
rat VR2      PTLSEDPSGPGITGNKKNP TSK-PGK---NSASEEDHLPLQVLQSP
                      ***.*****.*:..*:.* ** :.*****:***:

```

Fig. 7

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GAP of: ratvr2.pep check: 9190 from: 1 to: 554

ratVR2 Flrxb147g11

to: humanvr2.pep check: 5746 from: 1 to: 764

humanVR2 Flh21e11

Symbol comparison table: /usr/local/gog_9.1/gcgcore/data/rundata/blosum62.cmp
 CompCheck: 6430

Gap Weight: 12 Average Match: 2.912
 Length Weight: 4 Average Mismatch: -2.003

Quality: 2182 Length: 766
 Ratio: 3.939 Gaps: 4
 Percent Similarity: 81.703 Percent Identity: 79.167

Match display thresholds for the alignment(s) :

| = IDENTITY
 : = 2
 . = 1

ratvr2.pep x humanvr2.pep

```

      .           .           .           .           .
1  . . . . . STHASALSLAACTKQWDVVITYLLENPHQPASLEATDSLGN TVLH 44
      |||||
201 GQGTCFYFGELPLSLAACTKQWDVVSYLENPHQPASLQATDSQGNTVLH 250
      .           .           .           .           .
45 ALVMIADNSPENSALVIHMYDGLLQMGARLCPTVQLEEISNHQGLTPLKL 94
      |||||.||| ||| ||| ||||| ||||| ||||| ||||| |||||
251 ALVMISDNSAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKL 300
      .           .           .           .           .
95 AAKEGKIEIFRHILQREFSGPYQPLSRKFTEWCYGPVRVSLYDLSSVDSW 144
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
301 AAKEGKIEIFRHILQREFSG.LSHLSRKFTWCYGPVRVSLYDLASVDSC 349
      .           .           .           .           .
145 EKNSVLEIIAFHCKSPNRHRMVVLEPLNKLLQEKWDRLVSRPFFNFACYL 194
      |.||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
350 EENS VLEIIAFHCKSPHRHRMVVLEPLNKLLQAKWDLLIPKFFLNFLCNL 399
      .           .           .           .           .
195 VYMFIFTTVVAYHQPSLDQPAIPSSKATFGESMLLLGHILILLGGIYLLLG 244
      :||| ||| |||||. | . | | | | | | | | | | | | | | |
400 IYMFIFTAVAYHQPTLKKQAAPHLKAEVGNSMLLTGHILILLGGIYLLVG 449
  
```

Fig. 8

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245 QLWYFWRRRLFIWISFMDSYFEILFLLQALLTVLSQVLRFMETEWYLPLL 294
   ||||| . ||||| . ||||| ||||| . ||||| : |||||
450 QLWYFWRRHVFIWISFIDSYFEILFLFQALLTVVSQVLCFLAIEWYLPLL 499
   ||||| . ||||| ||||| ||||| ||||| ||||| ||||| : |||||
295 VLSVLGWLNLLEYTRGFQHTGIYSVMIQKVILRDLLRFLLVYLVFLFGF 344
   | . ||||| ||||| ||||| ||||| ||||| ||||| ||||| : |||||
500 VSALVLGWLNLLEYTRGFQHTGIYSVMIQKVILRDLLRFLLIYLVFLFGF 549
   ||||| . ||||| . ||||| . ||||| . ||||| : |||||
345 AVALVSLSREARSPKAPEDNNSTVTEQPTVGQEEE..PAPYRSILDASLE 392
   ||||| . ||||| . ||||| . ||||| . ||||| : |||||
550 AVALVSLSQEAWRPEAPTGPNATESVQPMEGQEDEGNGAQYRGILEASLE 599
   ||||| . ||||| . ||||| . ||||| . ||||| : |||||
393 LFKFTIGMGELAFQEQLRFRGVVLLLLLAYVLLTYVLLNMLIALMSETV 442
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| : |||||
600 LFKFTIGMGELAFQEQLHFRGMVLLLLLAYVLLTYILLNMLIALMSETV 649
   ||||| . ||||| ||||| ||||| ||||| ||||| ||||| : |||||
443 NHVADNSWSIWKLQKAISVLEMENGYWWCRRKKHREGRLKVGTRGDGTP 492
   ||||| . ||||| ||||| ||||| ||||| ||||| ||||| : |||||
650 NSVATDSWSIWKLQKAISVLEMENGYWWC.RKKQRAGVMLTVGTPDGSP 698
   ||||| . ||||| ||||| ||||| ||||| ||||| ||||| : |||||
493 DERWCFRVEEVNWAWEKTLPTLSEDPSPGITGNKKNPT....SKPGKN 538
   ||||| ||||| ||||| . ||||| ||||| ||||| : |||||
699 DERWCFRVEEVNWAWEQTLPTLCEDPSPGAGVPRTLENPVLASPPKEDED 748
   ||||| . ||||| ||||| ||||| ||||| ||||| : |||||
539 SASEEDHLPQLVQSP 554
   ||||| . ||||| ||||| ||||| ||||| ||||| : |||||
749 GASEENYVPVQLQSN 764

```

Fig. 8 continued

[illegible]

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1751 CAACATGGCCCTGGTGACCCTCCTGGTGGAGAACGAGCAGACGTCCAGG 1800
||||| | ||||| | ||||| | ||||| | ||||| |
716 GAACATGACGCTGGTGACCCTCTTGGTGGAGAATGGAGCAGATGTCCAGG 765
. . .
1801 CTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGGCGGCCTGGATTTC 1850
||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
766 CTGCGGGCTAACGGGGACTTCTTCAAGAAAACCAAAGGGAGGCCTGGCTTC 815
. . .
1851 TACTTCGGTGAAGTGCCTGCTGCCCCCTGGCCGCGTGCACCAACCAGCTGGG 1900
||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
816 TACTTTGGTGAGCTGCCCCCTGTCCCTGGCTGCGTGCACCAACCAGCTGGC 865
. . .
1901 CATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCG 1950
||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
866 CATTGTGAAGTTCCTGCTGCAGAACTCCTGGCAGCCTGCAGACATCAGCG 915
. . .
1951 CCAGGGACTCGGTGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGGCC 2000
|| ||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
916 CCCGGGACTCAGTGGGCAACACGGTGCTTCATGCCCTGGTGGAGGTGGCA 965
. . .
2001 GACAACACGGCCGACAACACGAAGTTTGTGACGAGCATGTACAATGAGAT 2050
|| ||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
966 GATAACACAGTTGACAACACCAAGTTCGTGACAAGCATGTACAACGAGAT 1015
. . .
2051 TCTGATGCTGGGGGCCAAACTGCACCCGACGCTGAAGCTGGAGGAGCTCA 2100
||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
1016 CTTGATCCTGGGGGCCAAACTCCACCCCACGCTGAAGCTGGAAGAGATCA 1065
. . .
2101 CCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCTGGGACCGGGAAG 2150
||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
1066 CCAACAGGAAGGGGCTCACGCCACTGGCTCTGGCTGCTAGCAGTGGGAAG 1115
. . .
2151 ATCGGGGTCTTGCCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAGTG 2200
||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
1116 ATCGGGGTCTTGCCCTACATTCTCCAGAGGGAGATCCATGAACCCGAGTG 1165
. . .
2201 CAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCCGTGCACT 2250
| ||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
1166 CCGACACCTATCCAGGAAGTTCACCGAATGGGCCTATGGGCCAGTGC ACT 1215
. . .
2251 CCTCGCTGTACGACCTGTCTGCATCGACACCTGCGAGAAGAACTCGGTG 2300
||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |
1216 CCTCCCTTTATGACCTGTCTGCATTGACACCTGTGAAAAGAACTCGGTT 1265

Fig. 9 continued

2301	CTGGAGGTGATCGCCTACAGCAGCAGCGAGACCCCTAATCGCCACGACAT	2350
1266	CTGGAGGTGATCGCTTACAGCAGCAGTGAGACCCCTAACCGTCATGACAT	1315
	.	.
2351	GCTCTTGGTGGAGCCGCTGAACCGACTCCTGCAGGACAAGTGGGACAGAT	2400
1316	GCTTCTCGTGGAACCCTTGAACCGACTCCTACAGGACAAGTGGGACAGAT	1365
	.	.
2401	TCGTCAAGCGCATCTTCTACTTTCAAATTCTTGGTCTACTGCCTGTACATG	2450
1366	TTGTCAAGCGCATCTTCTACTTTCAAATTCTTCGTCTACTGCTTGTATATG	1415
	.	.
2451	ATCATCTTCACCATGGCTGCCTACTACAGGCCCGTGGATGGCTTGCCTCC	2500
1416	ATCATCTTCACCGCGGCTGCCTACTATCGGCCTGTGGAAGGCTTGGCCCC	1465
	.	.
2501	CTTTAAGATGGAAAAAA... TTGGAGACTATTTCCGAGTTACTGGAGAGA	2547
1466	CTATAAGCTGAAAACACCGTTGGGGACTATTTCCGAGTCACCGGAGAGA	1515
	.	.
2548	T CCTGTCTGT GTTAGGAGGAGTCTACTTCTTTTTCCGAGGGATT CAGTAT	2597
1516	T CT TGTCTGT G TCAGGAGGAGTCTACTTCTTCTCCGAGGGATT CAATAT	1565
	.	.
2598	TTCCTGCAGAGGCGGCCGTCGATGAAGACCCTGTTTGTGGACAGCTACAG	2647
1566	TTCCTGCAGAGGCGACCATCCCTCAAGAGTTTGT TGTGGACAGCTACAG	1615
	.	.
2648	TGAGATGCTTTTCTTTCTGTCAGTCACTGTT CATGCTGGCCACCGTGGTGC	2697
1616	TGAGATACTTTTCTTTGTACAGTCGCTGTT CATGCTGGTGTCTGTGGTAC	1665
	.	.
2698	TGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTATTCTCCCCTG	2747
1666	TGTACTTCAGCCAACGCAAGGAGTATGTGGCTTCCATGGTGTCTCCCCTG	1715
	.	.
2748	GCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAGCAGAT	2797
1716	GCCATGGGCTGGACCAACATGCTCTACTATAACCCGAGGATTCCAGCAGAT	1765
	.	.
2798	GGGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCC	2847
1766	GGGCATCTATGCTGTGTCATGATTGAGAAGATGATCCTCAGAGACCTGTGCC	1815

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2848 GTTTCATGTTTGCTACATCGTCTTCTTGTTCCGGGTTTTCCACAGCGGTG 2897
| | | | | | | | | | | | | | | | | | | | | | | | | |
1816 GGTTTATGTTTCGTCTACCTCGTGTTCTTGTTTGGATTTCACAGCTGTG 1865

2898 GTGACGCTGATTGAAGACGGGAAGAATGACTCCCTGCCGTCTGAGTCCAC 2947
| | | | | | | | | | | | | | | | | | | | | | | | | |
1866 GTGACACTGATTGAGGATGGGAAGAATAACTCTCTGCCTATGGAGTCCAC 1915

2948 GTCGCACAGGTGGCGGGGGCCTGCCTGCAGGCCCCCGATAGCTCCTACA 2997
| | | | | | | | | | | | | | | | | | | | | | | | | |
1916 ACCACACAAGTGCCGGGGGTCTGCCTGCAAG...CCAGGTA ACTCTTACA 1962

2998 ACAGCCTGTACTCCACCTGCCTGGAGCTGTTCAAGTTCACCATCGGCATG 3047
| | | | | | | | | | | | | | | | | | | | | | | | | |
1963 ACAGCCTGTATTCCACATGTCTGGAGCTGTTCAAGTTCACCATCGGCATG 2012

3048 GCGGACCTGGAGTTCACTGAGAACTATGACTTCAAGGCTGTCTTCATCAT 3097
| | | | | | | | | | | | | | | | | | | | | | | | | |
2013 GCGGACCTGGAGTTCACTGAGAACTACGACTTCAAGGCTGTCTTCATCAT 2062

3098 CCTGCTGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGC 3147
| | | | | | | | | | | | | | | | | | | | | | | | | |
2063 CCTGTTACTGGCCTATGTGATTCTCACCTACATCCTTCTGCTCAACATGC 2112

3148 TCATCGCCCTCATGGGTGAGACTGTCAACAAGATCGCACAGGAGAGCAAG 3197
| | | | | | | | | | | | | | | | | | | | | | | | | |
2113 TCATTGCTCTCATGGGTGAGACCGTCAACAAGATTGCACAAGAGAGCAAG 2162

3198 AACATCTGGAAGCTGCAGAGAGCCATCACCATCCTGGACACGGAGAAGAG 3247
| | | | | | | | | | | | | | | | | | | | | | | | | |
2163 AACATCTGGAAGCTGCAGAGAGCCATCACCATCCTGGATACAGAGAAGAG 2212

3248 CTTCTTAAGTGCATGAGGAAGGCCTTCCGCTCAGGCAAGCTGCTGCAGG 3297
| | | | | | | | | | | | | | | | | | | | | | | | | |
2213 CTTCTGAAGTGCATGAGGAAGGCCTTCCGCTCTGGCAAGCTGCTGCAGG 2262

3298 TGGGGTACACACCTGATGGCAAGGACGACTACCGGTGGTGTTCAGGGTG 3347
| | | | | | | | | | | | | | | | | | | | | | | | | |
2263 TGGGGTTCCTCCTGACGGCAAGGATGACTACCGGTGGTGTTCAGGGTG 2312

3348 GACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCATCATCAACGA 3397
| | | | | | | | | | | | | | | | | | | | | | | | | |
2313 GACGAGGTAACTGGACTACCTGGAACACCAATGTGGGTATCATCAACGA 2362

Fig. 9 continued

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3398 AGACCCGGGCAACTGTGAGGGCGTCAAGCGCACCCCTGAGCTTCTCCCTGC 3447
||||| |||||||||||||||||||||||||||||||||||||||
2363 GGACCCAGGCAACTGTGAGGGCGTCAAGCGCACCCCTGAGCTTCTCCCTGA 2412

3448 GGTCAAGCAGAGTTTCAGGCAGACACTGGAAGAAGTTTGGCCCTGGTCCCC 3497
||||| || ||||||||||| ||| |||||||||||||||||||||||
2413 GGTCAAGCCGAGTTTCAGGGAGAACTGGAAGAAGTTTGGCCCTGGTTCCC 2462

3498 CTTTTAAGAGAGGCAAGTGCTCGAGATAGGCAGTCTGCTCAGCCCGAGGA 3547
||| || || || || || || || || || || || || || || || ||
2463 CTTCTGAGGGATGCAAGCACTCGAGATAGACATGCCACCCAGCAGGAAGA 2512

3548 AGTTTATCTGCGACAGTTTTCAGGGTCTCTGAAGCCAGAGGACGCTGAGG 3597
||||| || || || || || || || || || || || || || || || ||
2513 AGTTCAACTGAAGCATTATACGGGATCCCTTAAGCCAGAGGATGCTGAGG 2562

3598 TCTTCAAGAGTCCTGCCGCTTCCGGGGAGAAGTGA.GGACGTCACGCAGA 3646
| ||||| | | ||||||||| | | ||||| | |||||
2563 TTTTCAAGGATTCCATGGTCCCAGGGGAGAAATAATGGACACTATGCAGG 2612

3647 CAGCACTGTCAACACTGGGCCCTTAGGAGACCCCGTTGCCACGGGGGGCTG 3696
| || | | | | | | | | | | | | | | | | | | | |
2613 GATCAATG.....CGGGGTCTTTGGGTGGTCTG 2640

3697 CTGAGGGAACACCAGTGCTCTGTCAGCAGCCTGGCCTGGTCTGTGCCTGC 3746
|| ||||||| | | || | | | | | | | | | | | | | | |
2641 CTTAGGGGAAC.CAGCAGGGTTGACGTTATCTGGGTCCACTCTGTGCCTGC 2689

3747 CCA.GCATGTTCCCAAATCTGTGCTGGACAAGCTGTGGGAAGCGTTCTTG 3795
| || | | ||||| || | || | | | | | | | | | | | |
2690 CTAGGCACATTCTTAGGACTTCGGCGGGCCTGCTGTGGGAA.CTGGGAGG 2738

3796 GAAGCATGGGGAGTGATGTACATCCAACCGTCACTGTCCCAAGTGAATC 3845
| | | | | ||||| ||||||||| | | | | | | | | | |
2739 TGTGTGGGAATTGAGATGTGTATCCAACCATGA...TCTCCAAACATTTG 2785

3846 TCCTAACAGACTTTCAGGTTTTTACTCACTTTACTAAAAAAAAAAAAAAAA 3895
| | | | | ||||| || | || | | | | | | | | | | | |
2786 GCTTTCAACTCTTATGGACTTTATTAAACAGAGTGAATGGCAAATCTCT 2835

3896 AGGGCGGCCGCTTA 3909
| | | | |
2836 ACTTGGACACAT.. 2847

Fig. 9 continued

SUBSTITUTE SHEET (RULE 26)

```

      1 MKKWSSTDLGTAADPLQKDTCPDPLDGPNSRPPPAKPQLPTAKSRTRLF   50
        |.. .| | . | |...|.||..:||.:|| | : | : |||||
      1 MEQRASLDSEESESPPEQENSCLDPPDRDPNCKPPPVPKHIFTTRSRLF   50

    51 GKGDSEEAFVDCPHEEGELDSCPTITVSPVITIQRPGDGPTGARLLSQD   100
        ||||||| |. || |:| | | | | | | | : || || || | | | |
    51 GKGDSEEASPLDCPYEEGLASCPIITVSSVLTIQRPGDGPASVRPSSQD   100

   101 SVAASTEKTLRLYDRRSIFEAVAQNQCQDLESLLLFLQSKKKHLTDNEFK   150
        ||.| | | | | | | | | : || | . || : || | | | | | . ||
   101 SVSAG.EKPRLYDRRSIFDAVAQSNCQELESLLPFLQRSKKRLTDSEFK   149

   151 DPETGKTCLLKAMLNLDHGQNTTIPLLLEIARQTDSLKELVNASYTDSYY   200
        || || || || || || || || |. || | | | : || . || | | : || || || || ||
   150 DPETGKTCLLKAMLNLDHNGQNDTIALLLDVARKTDSLKFVNASYTDSYY   199

   201 KGOTALHIAIERRNMALVTLLVENGADVQAAAHGDFEKKTKGRPGFYFGE   250
        || || || || || || || || || || || || || || || || || || || || || || ||
   200 KGOTALHIAIERRNMTLVTLLVENGADVAAAANGDEFFKTKGRPGFYFGE   249

```

Fig. 10

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251 LPLSLAACTNQLGIVKFLQNSWQTADISARDSVGNTVLHALVEVADNTA 300
|||||
250 LPLSLAACTNQLAIVKFLQNSWQPADISARDSVGNTVLHALVEVADNTV 299
301 DNTKFVTSMYNEILMLGAKLHPTLKLEELTNKKGMTPLALAAGTGKIGVL 350
|||||
300 DNTKFVTSMYNEILILGAKLHPTLKLEETNRKGLTPLALAASSGKIGVL 349
351 AYILQREIQEPECRHLSRKFTWAYGPHSSLYDLSCIDTCEKNSVLEVI 400
|||||
350 AYILQREIHEPECRHLSRKFTWAYGPHSSLYDLSCIDTCEKNSVLEVI 399
401 AYSSSETPNRHDMLLVEPLNRLQDKWDRFVKRIFYFNFLVYCLYMIIFT 450
|||||
400 AYSSSETPNRHDMLLVEPLNRLQDKWDRFVKRIFYFNFFVYCLYMIIFT 449
451 MAAYYRPVDGLPPFKMEK.IGDYFRVTGEILSVLGGVYFFFRGIQYFLQR 499
|||||:||||:|. :|||||
450 AAAYYRPVEGLPPYKLNKNTVGDYFRVTGEILSVSGGVYFFFRGIQYFLQR 499
500 RPSMKTLFVDSYSEMLFFLQSLFMLATVVLYFSHLKEYVASMVFSALGW 549
|||:|.|||||.|||||.|||||
500 RPSLKSLFVDSYSEILFFVQSLFMLVSVVLYFSQRKEYVASMVFSAMGW 549
550 TNMLYYTRGFQQMGYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVTLI 599
|||||:|||||
550 TNMLYYTRGFQQMGYAVMIEKMILRDLCRFMFVYLVFLFGFSTAVVTLI 599
600 EDGKNDSLPESTSHRWGPACRPPDSSYNSLYSTCLELFKFTIGMGDLE 649
|||||.||| ||| : ||| : | .|||||
600 EDGKNNSLPMESTPHKCRGSACK.PGNSYNSLYSTCLELFKFTIGMGDLE 648
650 FTENYDFKAVFIILLLAYVILTYILLNMLIALMGETVKNKIAQESKNIWK 699
|||||
649 FTENYDFKAVFIILLLAYVILTYILLNMLIALMGETVKNKIAQESKNIWK 698
700 LQRAITILDTEKSFLKCMRKAFRSGKLLQVGYPDGKDDYRWCFRVDEVN 749
|||||:|||||
699 LQRAITILDTEKSFLKCMRKAFRSGKLLQVGFTPDGKDDYRWCFRVDEVN 748
750 WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSSRVSGRHWKNFALVPLLRE 799
|||||:|||||
749 WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSGRVSGRNWKNFALVPLLRD 798
800 ASARDRQSAQPEEVYLRQFSGSLKPDAEVFKSPAASGEK 839
|| ||| . | ||| : :. |||||
799 ASTRDRHATQQEEVQLKHYTGSLKPDAEVFKDSMVPGEK 838

Fig. 10 continued

SUBSTITUTE SHEET (RULE 26)

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CLUSTAL W (1.74) multiple sequence alignment

```

humanVR2.alt -----
human VR2      MTSPSSSPVFRLETLDGGQEDGSEADRGKLDGSGLPPEMESQFQGEDRKFPQIRVNLNY

humanVR2.alt -----
human VR2      RKGTGASQPDNRFDRDLFNAVSRGVPEDLAGLPEYLSKTSKYLTDSEYTEGSTGKTCL

humanVR2.alt -----
human VR2      MKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLQCVK

humanVR2.alt -----GRFFQKGQGTCTFYFGELPLSLAACTKQWDVVSYLENPHQPASLQA
human VR2      LLVENGANVHARACGRFFQKGQGTCTFYFGELPLSLAACTKQWDVVSYLENPHQPASLQA
                    *****

humanVR2.alt TDSQGN TVLHALVMISDNSAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKL
human VR2      TDSQGN TVLHALVMISDNSAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKL
                    *****

humanVR2.alt AAKEGKIEIFRHILQREFSGLSHLSRKFTWCYGPVVRVSLYDLASVDSCEENS VLEIIAF
human VR2      AAKEGKIEIFRHILQREFSGLSHLSRKFTWCYGPVVRVSLYDLASVDSCEENS VLEIIAF
                    *****

humanVR2.alt HCKSPHRHRMVVLEPLNKLQAKWDLLIPKFFDNFLCNLIYMFIFTAVAYHQPTLKKQAA
human VR2      HCKSPHRHRMVVLEPLNKLQAKWDLLIPKFFDNFLCNLIYMFIFTAVAYHQPTLKKQAA
                    *****

humanVR2.alt PHLKAEVGNSMLLTGHILILLGGIYLLVGQLWYFWRRHVFIWISFIDSYFEILFLFQALL
human VR2      PHLKAEVGNSMLLTGHYLILLGGIYLLVGQLWYFWRRHVFIWISFIDSYFEILFLFQALL
                    *****

humanVR2.alt TVVSQVLCFLAIEWYLPLLVSALVLGWLNL LYYTRGFQHTGIYSVMIQ-----
human VR2      TVVSQVLCFLAIEWYLPLLVSALVLGWLNL LYYTRGFQHTGIYSVMIQKVILRDLLRFL
                    *****

humanVR2.alt -----
human VR2      IYLVFLFGFAVALVSLSQEAWRPEAPTGPNATESVQPMEGQEDEGNGAQYRGILEASLEL

humanVR2.alt -----
human VR2      FKFTIGMGELAFQEQLHFRGMVLLLLLLAYVLLTYILLNMLIALMSETVNSVATDSWSIW

humanVR2.alt --KKAISVLEMENGYWWCRKKQRAGVMLTVGTPDGPDERWCFRVEEVN WASWEQTLPT
human VR2      KLQKKAISVLEMENGYWWCRKKQRAGVMLTVGTPDGPDERWCFRVEEVN WASWEQTLPT
                    :*****

humanVR2.alt LCEDPSGAGVPRTLENPVLASPPKEDEDGASEENYVPVQLLQSN
human VR2      LCEDPSGAGVPRTLENPVLASPPKEDEDGASEENYVPVQLLQSN
                    *****

```

Fig. 11

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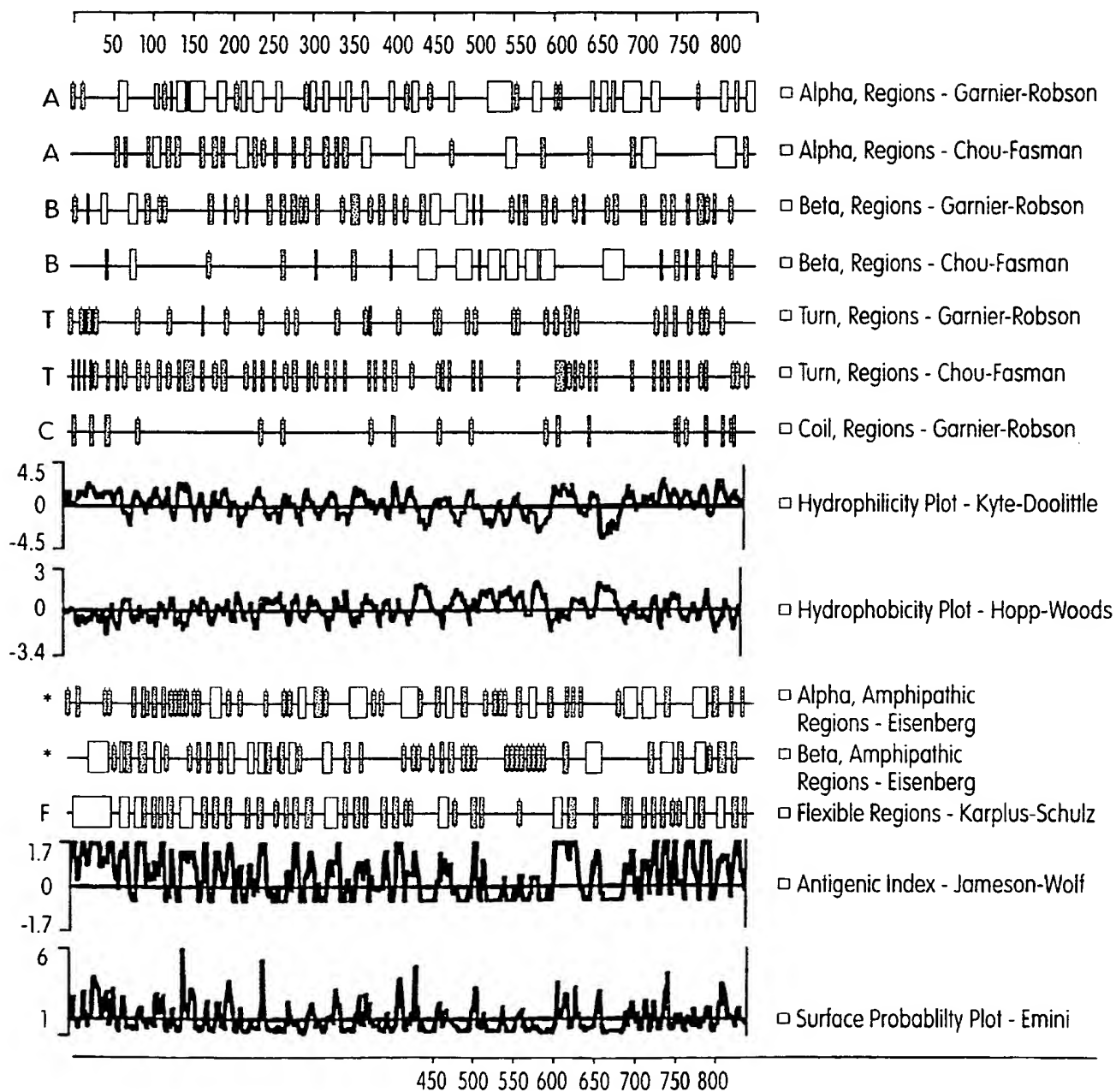


Fig. 12

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Protein Family / Domain Matches, HMMer version 2

Searching for complete domains

hmmpfam - search a single seq against HMM database

HMMER 2.1.1 (Dec 1998)

Copyright (C) 1992-1998 Washington University School of Medicine

HMMER is freely distributed under the GNU General Public License (GPL).

HMM file: /prod/ddm/sequal/PFAM/pfam4.2/PfamSequence file: /usr/ns-home/docs/sequal/orfanal/oa-script 18670 seq

Query: hVR-1

Scores for sequence family classification (score includes all domains):

Model	Description	Score	E-value	N
ank	Ank repeat	51.5	1.9e-11	3

Parsed for domains:

Model	Domain	seq-f	seq-t	hmm-f	hmm-t	score	E-value
ank	1/3	201	233 ..	1	33 []	34.4	2.6e-06
ank	2/3	248	283 ..	1	33 []	13.2	2
ank	3/3	333	361 ..	1	33 []	3.4	26

Alignments of top-scoring domains:

ank: domain 1 of 3, from 201 to 233: score 34.4, E = 2.6e-06

```

*->nGnTPLHlAarygnvevklLLehGAdvnartk<-*
      +G+T+LH+A +  n+ +v lL+e+GAdv a+

```

hVR-1	201	KGQTALHIAIERNMALVTLLVENGADVQAAAH	233
-------	-----	----------------------------------	-----

ank: domain 2 of 3, from 248 to 283: score 13.2, E = 2

```

*->nGnTPLHlAarygnvevklLLe...hGAdvnartk<-*
      G PL lAa ++++ +vk+LL+++ + Ad+ ar+

```

hVR-1	248	FGELPLSLAACTNQLGIVKFLlQnswQTADISARDS	283
-------	-----	--------------------------------------	-----

ank: domain 3 of 3, from 333 to 361: score 3.4, E = 26

```

*->nGnTPLHlAarygnvevklLLehGAdvnartk<-*
      +G TPL lAa +g++ v ++ L+      ++

```

hVR-1	333	KGMTPLALAAGTGKIGVLAYILQ----REIQEP	361
-------	-----	-----------------------------------	-----

Fig. 13

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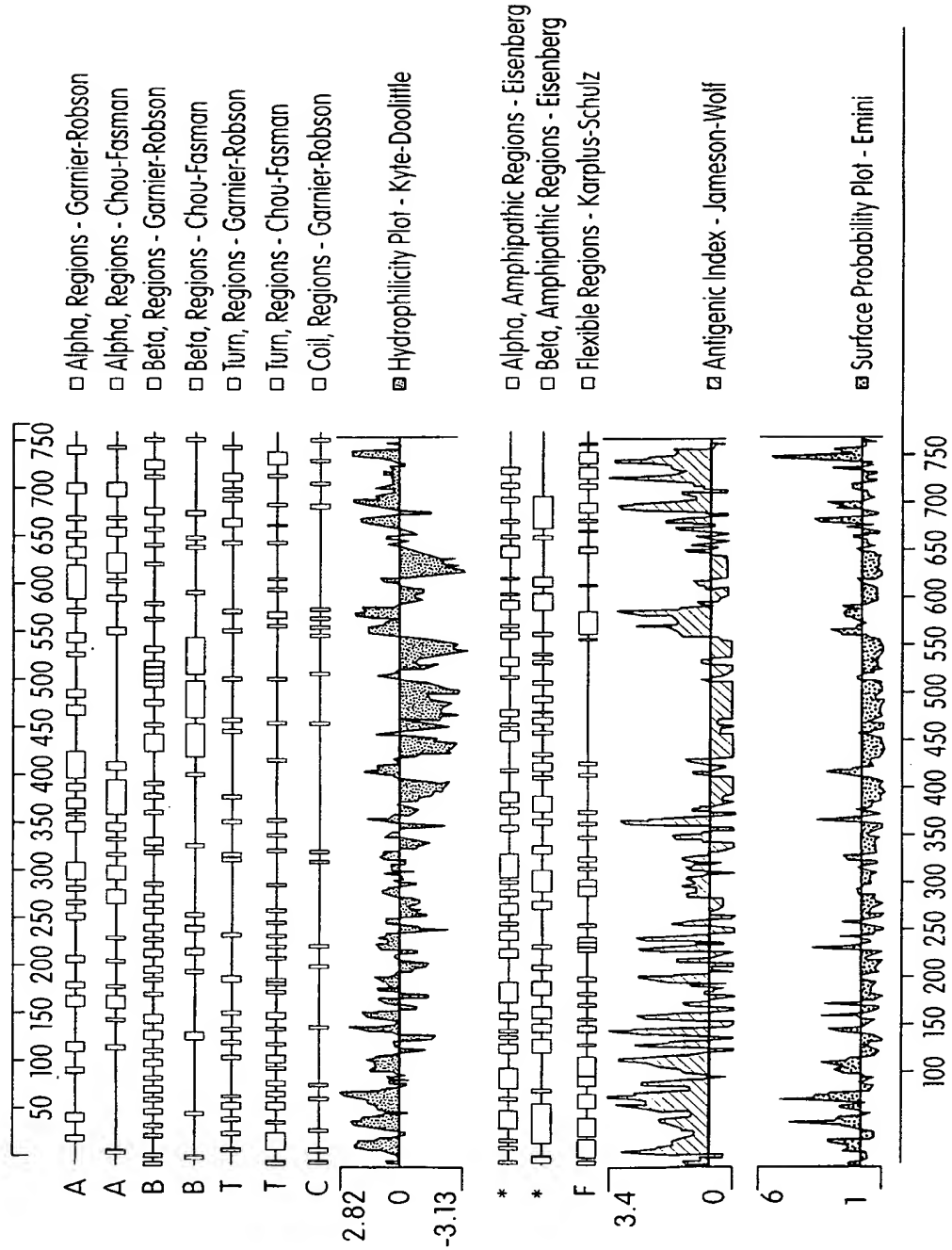


Fig. 14

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Protein Family / Domain Matches, HMMer version 2

Searching for complete domains

hmmpfam - search a single seq against HMM database

HMMER 2.1.1 (Dec 1998)

Copyright (C) 1992-1998 Washington University School of Medicine

HMMER is freely distributed under the GNU General Public License (GPL).

HMM file: /prod/ddm/seganal/PFAM/pfam4.2/Pfam
 Sequence file: /tmp/orfana1.5/g.aa

Query: Flh21e11

Scores for sequence family classification (score includes all domains):

Model	Description	Score	E-value	N
ank	PF00023 Ank repeat	53.7	4e-12	3

Parsed for domains:

Model	Domain	seq-f	seq-t	hmm-f	hmm-t	score	E-value
ank	1/3	162	194 ..	1	33 []	38.3	1.7e-07
ank	2/3	208	243 ..	1	33 []	6.4	4.3
ank	3/3	293	328 ..	1	33 []	8.8	2.1

Alignments of top-scoring domains:

ank: domain 1 of 3, from 162 to 194: score 38.3, E = 1.7e-07

->nGnTPLHLAarygnvevklLLehGAdvnartk<-
 +G+++LH+A ++ ++++vklL+e+GA+v+ar

Flh21e11 162 RGHSALHIAIEKRSLQCVKLLVENGANVHARAC 194

ank: domain 2 of 3, from 208 to 243: score 6.4, E = 4.3

->nGnTPLHLAarygnvavvklLLe...hGAdvnartk<-
 G PL lAa + +++vv +LLe++++ A+ a++

Flh21e11 208 FGELPLSLAACTKQWDVVSYLEnphQPASLQATDS 243

ank: domain 3 of 3, from 293 to 328: score 8.8, E = 2.1

->nGnTPLHLAarygnvevklLLe...hGAdvnartk<-
 + +TPL lAa++g++e+ + L+++ G + +r

Flh21e11 293 QDLTPLKLAAKEQKLEIFRHILQrafSGLSHLSRK 328

Fig. 15

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>hvr2.altFL (full-length predicted)
 MTSPSSSPVFRLETLGGQEDGSEADRGKLDGSGLPPEMQFQGEDRKFAPIRVNLNY
 RKG TGASQDPNRFDRDRILFNAVSRGVPEDLAGLPEYLSKTSKYLTDSEYTEGSTGKTCL
 MKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLQCVK
 LLVENGANVHARACGRFFQKGQGTCTFYFGEPLSLAACTKQWDVVSYLLENPHQPASLQATSQGNTVLHALVM
 ISDNSAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKLAKEGKIEIFRHIL
 QREFSGLSHLSRKFTWCYGPVRVSLYDLASVDSCEENSIVLEIIAFHCKSPHRHRMVVLE
 PLNKLLOAKWDDLIPKFFLNFLCNLIYMFIFTAVAYHQPTLKKQAAPHLKAEVGNSMLLT
 GHILILLGGIYLLVGQLWYFWRHHVFIWISFIDSYFEILFLFQALLTVVSQVLCFLAIEW
 YLPLLVSALVLGWLNLLEYTRGFQHTGIYSVMIQKKAISVLEMENGYWWCRKKQRAGVML
 TVGTKPDGSPDERWCFRVEEVNWSWEQTLPTLCEDPGAGVPRTLENPVLASPPKEDED
 GASEENYVPVQLQSN

Fig. 16

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CLUSTAL W (1.74) multiple sequence alignment

```

humanVR2      MTSPSSSPVFRLETLDGGQEDGSEADRGKLDGSGGLPPMESQFQGEDRKFAPQIRVNLNY
hVR2.altfL    MTSPSSSPVFRLETLDGGQEDGSEADRGKLDGSGGLPPMESQFQGEDRKFAPQIRVNLNY
*****

humanVR2      RKGTGASQPDPNRFDRDLFNAVSRGVPEDLAGLPEYLSKTSKYLTDSEYTEGSTGKTCL
hVR2.altfL    RKGTGASQPDPNRFDRDLFNAVSRGVPEDLAGLPEYLSKTSKYLTDSEYTEGSTGKTCL
*****

humanVR2      MKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLQCVK
hVR2.altfL    MKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLQCVK
*****

humanVR2      LLVENGANVHARACGRFFQKGQGTCTFYFGELPLSLAACTKQWDVVSYLLENPHQPASLQA
hVR2.altfL    LLVENGANVHARACGRFFQKGQGTCTFYFGELPLSLAACTKQWDVVSYLLENPHQPASLQA
*****

humanVR2      TDSQGNTVLHALVMISDNSAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKL
hVR2.altfL    TDSQGNTVLHALVMISDNSAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKL
*****

humanVR2      AAKEGKIEIFRHILQREFSGLSHLSRKFTWCYGPVRVSLYDLASVDSCEENSVLEIIAF
hVR2.altfL    AAKEGKIEIFRHILQREFSGLSHLSRKFTWCYGPVRVSLYDLASVDSCEENSVLEIIAF
*****

humanVR2      HCKSPHRHRMVVLEPLNKLQAKWDLIPKFFLNFLCNLIYMFIFTAVAYHQPTLKKQAA
hVR2.altfL    HCKSPHRHRMVVLEPLNKLQAKWDLIPKFFLNFLCNLIYMFIFTAVAYHQPTLKKQAA
*****

humanVR2      PHLKAEVGNMMLLTGHILILLGGIYLLVGQLWYFWRRHVFIWISFIDSYEILFLFQALL
hVR2.altfL    PHLKAEVGNMMLLTGHILILLGGIYLLVGQLWYFWRRHVFIWISFIDSYEILFLFQALL
*****

humanVR2      TVVSQVLCFLAIEWYLPLLVSALVLGWLNLNLLYYTRGFQHTGIYSVMIQKVLRLDLLRFL
hVR2.altfL    TVVSQVLCFLAIEWYLPLLVSALVLGWLNLNLLYYTRGFQHTGIYSVMIQK-----
*****

humanVR2      IYLVFLFGFAVALVSLSQEAWRPEAPTGNATESVQPMEGQEDGNGAQYRGILEASLEL
hVR2.altfL    -----

humanVR2      FKFTIGMGELAFQEQLHFRGMVLLLLLAYVLLTYILLNMLIALMSETVNSVATDSWSIW
hVR2.altfL    -----

humanVR2      KLQKAISVLEMENGYWWCRKKQRAGVMLTVGTPDGSPDERWCFRVEEVNWSWEQTLPT
hVR2.altfL    ---KAISVLEMENGYWWCRKKQRAGVMLTVGTPDGSPDERWCFRVEEVNWSWEQTLPT
*****

humanVR2      LCEDPSGAGVPRTLNPFVLASPPKEDEDGASEENYVPVQLLQSN
hVR2.altfL    LCEDPSGAGVPRTLNPFVLASPPKEDEDGASEENYVPVQLLQSN
*****

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Fig. 17

- 1 -

SEQUENCE LISTING

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Thr Asp Ser Leu Lys Glu Leu Val Asn Ala Ser Tyr Thr Asp Ser Tyr	
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Glu Pro Glu Cys Arg His Leu Ser Arg Lys Phe Thr Glu Trp Ala Tyr	
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gag aag aac tcg gtg ctg gag gtg atc gcc tac agc agc agc gag acc	2333
Glu Lys Asn Ser Val Leu Glu Val Ile Ala Tyr Ser Ser Ser Glu Thr	

- 4 -

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Pro Asn Arg His Asp Met Leu Leu Val Glu Pro Leu Asn Arg Leu Leu			
410	415	420	
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Gln Asp Lys Trp Asp Arg Phe Val Lys Arg Ile Phe Tyr Phe Asn Phe			
425	430	435	
ctg gtc tac tgc ctg tac atg atc atc ttc acc atg gct gcc tac tac			2477
Leu Val Tyr Cys Leu Tyr Met Ile Ile Phe Thr Met Ala Ala Tyr Tyr			
440	445	450	455
agg ccc gtg gat ggc ttg cct ccc ttt aag atg gaa aaa att gga gac			2525
Arg Pro Val Asp Gly Leu Pro Pro Phe Lys Met Glu Lys Ile Gly Asp			
460	465	470	
tat ttc cga gtt act gga gag atc ctg tct gtg tta gga gga gtc tac			2573
Tyr Phe Arg Val Thr Gly Glu Ile Leu Ser Val Leu Gly Gly Val Tyr			
475	480	485	
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Phe Phe Phe Arg Gly Ile Gln Tyr Phe Leu Gln Arg Arg Pro Ser Met			
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Lys Thr Leu Phe Val Asp Ser Tyr Ser Glu Met Leu Phe Phe Leu Gln			
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Ser Leu Phe Met Leu Ala Thr Val Val Leu Tyr Phe Ser His Leu Lys			
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gag tat gtg gct tcc atg gta ttc tcc ctg gcc ttg ggc tgg acc aac			2765
Glu Tyr Val Ala Ser Met Val Phe Ser Leu Ala Leu Gly Trp Thr Asn			
540	545	550	
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Met Leu Tyr Tyr Thr Arg Gly Phe Gln Gln Met Gly Ile Tyr Ala Val			
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Met Ile Glu Lys Met Ile Leu Arg Asp Leu Cys Arg Phe Met Phe Val			
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Tyr Ile Val Phe Leu Phe Gly Phe Ser Thr Ala Val Val Thr Leu Ile			
585	590	595	
gaa gac ggg aag aat gac tcc ctg ccg tct gag tcc acg tcg cac agg			2957
Glu Asp Gly Lys Asn Asp Ser Leu Pro Ser Glu Ser Thr Ser His Arg			
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tgg cgg ggg cct gcc tgc agg ccc ccc gat agc tcc tac aac agc ctg			3005
Trp Arg Gly Pro Ala Cys Arg Pro Pro Asp Ser Ser Tyr Asn Ser Leu			
620	625	630	

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Tyr Ser Thr Cys Leu Glu Leu Phe Lys Phe Thr Ile Gly Met Gly Asp	
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Leu Glu Phe Thr Glu Asn Tyr Asp Phe Lys Ala Val Phe Ile Ile Leu	
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Leu Leu Ala Tyr Val Ile Leu Thr Tyr Ile Leu Leu Leu Asn Met Leu	
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Ile Ala Leu Met Gly Glu Thr Val Asn Lys Ile Ala Gln Glu Ser Lys	
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Asn Ile Trp Lys Leu Gln Arg Ala Ile Thr Ile Leu Asp Thr Glu Lys	
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Ser Phe Leu Lys Cys Met Arg Lys Ala Phe Arg Ser Gly Lys Leu Leu	
715 720 725	
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Gln Val Gly Tyr Thr Pro Asp Gly Lys Asp Asp Tyr Arg Trp Cys Phe	
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Arg Val Asp Glu Val Asn Trp Thr Thr Trp Asn Thr Asn Val Gly Ile	
745 750 755	
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Ile Asn Glu Asp Pro Gly Asn Cys Glu Gly Val Lys Arg Thr Leu Ser	
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Phe Ser Leu Arg Ser Ser Arg Val Ser Gly Arg His Trp Lys Asn Phe	
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gcc ctg gtc ccc ctt tta aga gag gca agt gct cga gat agg cag tct	3533
Ala Leu Val Pro Leu Leu Arg Glu Ala Ser Ala Arg Asp Arg Gln Ser	
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gct cag ccc gag gaa gtt tat ctg cga cag ttt tca ggg tct ctg aag	3581
Ala Gln Pro Glu Glu Val Tyr Leu Arg Gln Phe Ser Gly Ser Leu Lys	
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cca gag gac gct gag gtc ttc aag agt cct gcc gct tcc ggg gag aag	3629
Pro Glu Asp Ala Glu Val Phe Lys Ser Pro Ala Ala Ser Gly Glu Lys	
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Pro	Pro	Pro	Ala	Lys	Pro	Gln	Leu	Pro	Thr	Ala	Lys	Ser	Arg	Thr	Arg
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Leu	Phe	Gly	Lys	Gly	Asp	Ser	Glu	Glu	Ala	Phe	Pro	Val	Asp	Cys	Pro
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His	Glu	Glu	Gly	Glu	Leu	Asp	Ser	Cys	Pro	Thr	Ile	Thr	Val	Ser	Pro
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Val	Ile	Thr	Ile	Gln	Arg	Pro	Gly	Asp	Gly	Pro	Thr	Gly	Ala	Arg	Leu
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Leu	Ser	Gln	Asp	Ser	Val	Ala	Ala	Ser	Thr	Glu	Lys	Thr	Leu	Arg	Leu
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Tyr	Asp	Arg	Arg	Ser	Ile	Phe	Glu	Ala	Val	Ala	Gln	Asn	Asn	Cys	Gln
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Asp	Leu	Glu	Ser	Leu	Leu	Leu	Phe	Leu	Gln	Lys	Ser	Lys	Lys	His	Leu
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Thr	Asp	Asn	Glu	Phe	Lys	Asp	Pro	Glu	Thr	Gly	Lys	Thr	Cys	Leu	Leu
145					150					155				160	

Lys	Ala	Met	Leu	Asn	Leu	His	Asp	Gly	Gln	Asn	Thr	Thr	Ile	Pro	Leu
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Leu	Leu	Glu	Ile	Ala	Arg	Gln	Thr	Asp	Ser	Leu	Lys	Glu	Leu	Val	Asn
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Ala	Ser	Tyr	Thr	Asp	Ser	Tyr	Tyr	Lys	Gly	Gln	Thr	Ala	Leu	His	Ile
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Ala	Ile	Glu	Arg	Arg	Asn	Met	Ala	Leu	Val	Thr	Leu	Leu	Val	Glu	Asn
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 Ala Cys Thr Asn Gln Leu Gly Ile Val Lys Phe Leu Leu Gln Asn Ser
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 Trp Gln Thr Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val
 275 280 285
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 Phe Val Thr Ser Met Tyr Asn Glu Ile Leu Met Leu Gly Ala Lys Leu
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 His Pro Thr Leu Lys Leu Glu Glu Leu Thr Asn Lys Lys Gly Met Thr
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 Pro Leu Ala Leu Ala Ala Gly Thr Gly Lys Ile Gly Val Leu Ala Tyr
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 Ile Leu Gln Arg Glu Ile Gln Glu Pro Glu Cys Arg His Leu Ser Arg
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 Lys Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp
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 Ala Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val
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 Arg Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile
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 Phe Thr Met Ala Ala Tyr Tyr Arg Pro Val Asp Gly Leu Pro Pro Phe
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 Lys Met Glu Lys Ile Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu
 465 470 475 480
 Ser Val Leu Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe
 485 490 495
 Leu Gln Arg Arg Pro Ser Met Lys Thr Leu Phe Val Asp Ser Tyr Ser
 500 505 510
 Glu Met Leu Phe Phe Leu Gln Ser Leu Phe Met Leu Ala Thr Val Val
 515 520 525
 Leu Tyr Phe Ser His Leu Lys Glu Tyr Val Ala Ser Met Val Phe Ser

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Gln Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp		
	565	570 575
Leu Cys Arg Phe Met Phe Val Tyr Ile Val Phe Leu Phe Gly Phe Ser		
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Thr Ala Val Val Thr Leu Ile Glu Asp Gly Lys Asn Asp Ser Leu Pro		
	595	600 605
Ser Glu Ser Thr Ser His Arg Trp Arg Gly Pro Ala Cys Arg Pro Pro		
	610	615 620
Asp Ser Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys		
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Phe Thr Ile Gly Met Gly Asp Leu Glu Phe Thr Glu Asn Tyr Asp Phe		
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Lys Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr		
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Ile Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Asn		
	675	680 685
Lys Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile		
690	695	700
Thr Ile Leu Asp Thr Glu Lys Ser Phe Leu Lys Cys Met Arg Lys Ala		
705	710	715 720
Phe Arg Ser Gly Lys Leu Leu Gln Val Gly Tyr Thr Pro Asp Gly Lys		
	725	730 735
Asp Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr		
	740	745 750
Trp Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu		
	755	760 765
Gly Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser		
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Gly Arg His Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Glu Ala		
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Ser Ala Arg Asp Arg Gln Ser Ala Gln Pro Glu Glu Val Tyr Leu Arg		
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caa aag gac acc tgc cca gac ccc ctg gat gga gac cct aac tcc agg 96
 Gln Lys Asp Thr Cys Pro Asp Pro Leu Asp Gly Asp Pro Asn Ser Arg
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cca cct cca gcc aag ccc cag ctc ccc acg gcc aag agc cgc acc cgg 144
 Pro Pro Pro Ala Lys Pro Gln Leu Pro Thr Ala Lys Ser Arg Thr Arg
 35 40 45

ctc ttt ggg aag ggt gac tcg gag gag gct ttc ccg gtg gat tgc ccc 192
 Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Phe Pro Val Asp Cys Pro
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cac gag gaa ggt gag ttg gac tcc tgc ccg acc atc aca gtc agc cct 240
 His Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro
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 Val Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu
 85 90 95

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 Leu Ser Gln Asp Ser Val Ala Ala Ser Thr Glu Lys Thr Leu Arg Leu
 100 105 110

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 Tyr Asp Arg Arg Ser Ile Phe Glu Ala Val Ala Gln Asn Asn Cys Gln
 115 120 125

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 Asp Leu Glu Ser Leu Leu Phe Leu Gln Lys Ser Lys Lys His Leu
 130 135 140

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 Thr Asp Asn Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu
 145 150 155 160

aaa gcc atg ctc aac ctg cac gac gga cag aac acc acc atc ccc ctg 528
 Lys Ala Met Leu Asn Leu His Asp Gly Gln Asn Thr Thr Ile Pro Leu
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ctc ctg gag atc gcg cgg caa acg gac agc ctg aag gag ctt gtc aac 576

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Leu	Leu	Glu	Ile	Ala	Arg	Gln	Thr	Asp	Ser	Leu	Lys	Glu	Leu	Val	Asn	
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gcc	agc	tac	acg	gac	agc	tac	tac	aag	ggc	cag	aca	gca	ctg	cac	atc	624
Ala	Ser	Tyr	Thr	Asp	Ser	Tyr	Tyr	Lys	Gly	Gln	Thr	Ala	Leu	His	Ile	
		195					200					205				
gcc	atc	gag	aga	cgc	aac	atg	gcc	ctg	gtg	acc	ctc	ctg	gtg	gag	aac	672
Ala	Ile	Glu	Arg	Arg	Asn	Met	Ala	Leu	Val	Thr	Leu	Leu	Val	Glu	Asn	
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Gly	Ala	Asp	Val	Gln	Ala	Ala	Ala	His	Gly	Asp	Phe	Phe	Lys	Lys	Thr	
225					230				235					240		
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Lys	Gly	Arg	Pro	Gly	Phe	Tyr	Phe	Gly	Glu	Leu	Pro	Leu	Ser	Leu	Ala	
				245					250					255		
gcg	tgc	acc	aac	cag	ctg	ggc	atc	gtg	aag	ttc	ctg	ctg	cag	aac	tcc	816
Ala	Cys	Thr	Asn	Gln	Leu	Gly	Ile	Val	Lys	Phe	Leu	Leu	Gln	Asn	Ser	
			260					265					270			
tgg	cag	acg	gcc	gac	atc	agc	gcc	agg	gac	tgc	gtg	ggc	aac	acg	gtg	864
Trp	Gln	Thr	Ala	Asp	Ile	Ser	Ala	Arg	Asp	Ser	Val	Gly	Asn	Thr	Val	
		275					280					285				
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Leu	His	Ala	Leu	Val	Glu	Val	Ala	Asp	Asn	Thr	Ala	Asp	Asn	Thr	Lys	
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Phe	Val	Thr	Ser	Met	Tyr	Asn	Glu	Ile	Leu	Met	Leu	Gly	Ala	Lys	Leu	
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His	Pro	Thr	Leu	Lys	Leu	Glu	Glu	Leu	Thr	Asn	Lys	Lys	Gly	Met	Thr	
				325					330					335		
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Pro	Leu	Ala	Leu	Ala	Ala	Gly	Thr	Gly	Lys	Ile	Gly	Val	Leu	Ala	Tyr	
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Ile	Leu	Gln	Arg	Glu	Ile	Gln	Glu	Pro	Glu	Cys	Arg	His	Leu	Ser	Arg	
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Lys	Phe	Thr	Glu	Trp	Ala	Tyr	Gly	Pro	Val	His	Ser	Ser	Leu	Tyr	Asp	
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Leu	Ser	Cys	Ile	Asp	Thr	Cys	Glu	Lys	Asn	Ser	Val	Leu	Glu	Val	Ile	
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Ala	Tyr	Ser	Ser	Ser	Glu	Thr	Pro	Asn	Arg	His	Asp	Met	Leu	Leu	Val	
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Arg Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile	
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Ile Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Asn		
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Trp Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu		
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Gly Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser		
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Gln Phe Ser Gly Ser Leu Lys Pro Glu Asp Ala Glu Val Phe Lys Ser		
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Pro Ala Ala Ser Gly Glu Lys		
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Asn Ala Val Ser Arg Gly Val Pro Glu Asp Leu Ala Gly Leu Pro Glu
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Tyr Leu Ser Lys Thr Ser Lys Tyr Leu Thr Asp Ser Glu Tyr Thr Glu
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Gly Ser Thr Gly Lys Thr Cys Leu Met Lys Ala Val Leu Asn Leu Lys
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Asp Gly Val Asn Ala Cys Ile Leu Pro Leu Leu Gln Ile Asp Arg Asp
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Ser Gly Asn Pro Gln Pro Leu Val Asn Ala Gln Cys Thr Asp Asp Tyr
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Lys Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu	
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Leu Thr Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val	
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Glu Leu Ala Phe Gln Glu Leu His Phe Arg Gly Met Val Leu Leu	
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Trp Ser Ile Trp Lys Leu Gln Lys Ala Ile Ser Val Leu Glu Met Glu
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acc gtt gcc act aag cca gat ggc agc ccg gat gag cgc tgg tgc ttc 2472
Thr Val Gly Thr Lys Pro Asp Gly Ser Pro Asp Glu Arg Trp Cys Phe
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Leu Cys Glu Asp Pro Ser Gly Ala Gly Val Pro Arg Thr Leu Glu Asn
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Pro Val Leu Ala Ser Pro Pro Lys Glu Asp Glu Asp Gly Ala Ser Glu
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 Ser Gly Asn Pro Gln Pro Leu Val Asn Ala Gln Cys Thr Asp Asp Tyr
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 Tyr Arg Gly His Ser Ala Leu His Ile Ala Ile Glu Lys Arg Ser Leu
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 Ala Cys Gly Arg Phe Phe Gln Lys Gly Gln Gly Thr Cys Phe Tyr Phe
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 Val Ser Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala
 225 230 235 240
 Thr Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser
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 Asp Asn Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly
 260 265 270
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 Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly
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 Lys Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu
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 Phe Ile Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu
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 Glu Leu Ala Phe Gln Glu Gln Leu His Phe Arg Gly Met Val Leu Leu
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 Gly Ser Gly Leu Pro Pro Met Glu Ser Gln Phe Gln Gly Glu Asp Arg
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agg gtg gag gag gtg aac tgg gct tca tgg gag cag acg ctg cct acg Arg Val Glu Glu Val Asn Trp Ala Ser Trp Glu Gln Thr Leu Pro Thr 705 710 715 720			2160
ctg tgt gag gac ccg tca ggg gca ggt gtc cct cga act ctc gag aac Leu Cys Glu Asp Pro Ser Gly Ala Gly Val Pro Arg Thr Leu Glu Asn 725 730 735			2208
cct gtc ctg gct tcc cct ccc aag gag gat gag gat ggt gcc tct gag Pro Val Leu Ala Ser Pro Pro Lys Glu Asp Glu Asp Gly Ala Ser Glu 740 745 750			2256
gaa aac tat gtg ccc gtc cag ctc ctc cag tcc aac Glu Asn Tyr Val Pro Val Gln Leu Leu Gln Ser Asn 755 760			2292

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<400> 7

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1 5 10 15	
gag cta ccc ctc tct ttg gcc gct tgc acc aag cag tgg gat gtg gta	95
Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val Val	
20 25 30	
agc tac ctc ctg gag aac cca cac cag ccc gcc agc ctg cag gcc act	143
Ser Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala Thr	
35 40 45	
gac tcc cag ggc aac aca gtc ctg cat gcc cta gtg atg atc tcg gac	191
Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser Asp	
50 55 60	
aac tca gct gag aac att gca ctg gtg acc agc atg tat gat ggg ctc	239
Asn Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly Leu	
65 70 75	
ctc caa gct ggg gcc cgc ctc tgc cct acc gtg cag ctt gag gac atc	287
Leu Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp Ile	
80 85 90 95	
cgc aac ctg cag gat ctc acg cct ctg aag ctg gcc gcc aag gag ggc	335
Arg Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu Gly	
100 105 110	
aag atc gag att ttc agg cac atc ctg cag cgg gag ttt tca gga ctg	383
Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly Leu	
115 120 125	
agc cac ctt tcc cga aag ttc acc gag tgg tgc tat ggg cct gtc cgg	431
Ser His Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr Gly Pro Val Arg	
130 135 140	
gtg tcg ctg tat gac ctg gct tct gtg gac agc tgt gag gag aac tca	479
Val Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn Ser	
145 150 155	
gtg ctg gag atc att gcc ttt cat tgc aag agc ccg cac cga cac cga	527
Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His Arg	
160 165 170 175	
atg gtc gtt ttg gag ccc ctg aac aaa ctg ctg cag gcg aaa tgg gat	575
Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp Asp	
180 185 190	
ctg ctc atc ccc aag ttc ttc tta aac ttc ctg tgt aat ctg atc tac	623
Leu Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile Tyr	
195 200 205	
atg ttc atc ttc acc gct gtt gcc tac cat cag cct acc ctg aag aag	671
Met Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys Lys	
210 215 220	
cag gcc gcc cct cac ctg aaa gcg gag gtt gga aac tcc atg ctg ctg	719
Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu Leu	

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225	230	235	
acg ggc cac atc ctt atc ctg cta ggg ggg atc tac ctc ctc gtg ggc Thr Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val Gly 240 245 250 255			767
cag ctg tgg tac ttc tgg cgg cgc cac gtg ttc atc tgg atc tgg ttc Gln Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Ser Phe 260 265 270			815
ata gac agc tac ttt gaa atc ctc ttc ctg ttc cag gcc ctg ctc aca Ile Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu Thr 275 280 285			863
gtg gtg tcc cag gtg ctg tgt ttc ctg gcc atc gag tgg tac ctg ccc Val Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu Pro 290 295 300			911
ctg ctt gtg tct gcg ctg gtg ctg ggc tgg ctg aac ctg ctt tac tat Leu Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr Tyr 305 310 315			959
aca cgt ggc ttc cag cac aca ggc atc tac agt gtc atg atc cag aag Thr Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln Lys 320 325 330 335			1007
aaa gcc atc tct gtc ctg gag atg gag aat ggc tat tgg tgg tgc agg Lys Ala Ile Ser Val Leu Glu Met Glu Asn Gly Tyr Trp Trp Cys Arg 340 345 350			1055
aag aag cag cgg gca ggt gtg atg ctg acc gtt ggc act aag cca gat Lys Lys Gln Arg Ala Gly Val Met Leu Thr Val Gly Thr Lys Pro Asp 355 360 365			1103
ggc agc ccg gat gag cgc tgg tgc ttc agg gtg gag gag gtg aac tgg Gly Ser Pro Asp Glu Arg Trp Cys Phe Arg Val Glu Glu Val Asn Trp 370 375 380			1151
gct tca tgg gag cag acg ctg cct acg ctg tgt gag gac ccg tca ggg Ala Ser Trp Glu Gln Thr Leu Pro Thr Leu Cys Glu Asp Pro Ser Gly 385 390 395			1199
gca ggt gtc cct cga act ctc gag aac cct gtc ctg gct tcc cct ccc Ala Gly Val Pro Arg Thr Leu Glu Asn Pro Val Leu Ala Ser Pro Pro 400 405 410 415			1247
aag gag gat gag gat ggt gcc tct gag gaa aac tat gtg ccc gtc cag Lys Glu Asp Glu Asp Gly Ala Ser Glu Glu Asn Tyr Val Pro Val Gln 420 425 430			1295
ctc ctc cag tcc aac tgatggccca gatgcagcag gaggccagag gacagagcag Leu Leu Gln Ser Asn 435			1350
aggatctttc caaccacatc tgctggctct ggggtcccag tgaattctgg tggcaaatat			1410
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agcggacgcg tgggtcgac

1489

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<211> 436

<212> PRT

<213> Homo sapiens

<400> 8

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 20 25 30

Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala Thr Asp
 35 40 45

Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser Asp Asn
 50 55 60

Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly Leu Leu
 65 70 75 80

Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp Ile Arg
 85 90 95

Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu Gly Lys
 100 105 110

Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly Leu Ser
 115 120 125

His Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr Gly Pro Val Arg Val
 130 135 140

Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn Ser Val
 145 150 155 160

Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His Arg Met
 165 170 175

Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp Asp Leu
 180 185 190

Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile Tyr Met
 195 200 205

Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys Lys Gln
 210 215 220

Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu Leu Thr
 225 230 235 240

Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val Gly Gln
 245 250 255

Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Ser Phe Ile

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260	265	270	
Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu Thr Val			
275	280	285	
Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu Pro Leu			
290	295	300	
Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr Tyr Thr			
305	310	315	320
Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln Lys Lys			
	325	330	335
Ala Ile Ser Val Leu Glu Met Glu Asn Gly Tyr Trp Trp Cys Arg Lys			
	340	345	350
Lys Gln Arg Ala Gly Val Met Leu Thr Val Gly Thr Lys Pro Asp Gly			
	355	360	365
Ser Pro Asp Glu Arg Trp Cys Phe Arg Val Glu Glu Val Asn Trp Ala			
	370	375	380
Ser Trp Glu Gln Thr Leu Pro Thr Leu Cys Glu Asp Pro Ser Gly Ala			
385	390	395	400
Gly Val Pro Arg Thr Leu Glu Asn Pro Val Leu Ala Ser Pro Pro Lys			
	405	410	415
Glu Asp Glu Asp Gly Ala Ser Glu Glu Asn Tyr Val Pro Val Gln Leu			
	420	425	430
Leu Gln Ser Asn			
435			
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1	5	10	15
cta ccc ctc tct ttg gcc gct tgc acc aag cag tgg gat gtg gta agc			
Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val Val Ser			96
	20	25	30
tac ctc ctg gag aac cca cac cag ccc gcc agc ctg cag gcc act gac			144
Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala Thr Asp			
	35	40	45

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tcc cag ggc aac aca gtc ctg cat gcc cta gtg atg atc tcg gac aac	192
Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser Asp Asn	
50 55 60	
tca gct gag aac att gca ctg gtg acc agc atg tat gat ggg ctc ctc	240
Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly Leu Leu	
65 70 75 80	
caa gct ggg gcc cgc ctc tgc cct acc gtg cag ctt gag gac atc cgc	288
Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp Ile Arg	
85 90 95	
aac ctg cag gat ctc acg cct ctg aag ctg gcc gcc aag gag ggc aag	336
Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu Gly Lys	
100 105 110	
atc gag att ttc agg cac atc ctg cag cgg gag ttt tca gga ctg agc	384
Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly Leu Ser	
115 120 125	
cac ctt tcc cga aag ttc acc gag tgg tgc tat ggg cct gtc cgg gtg	432
His Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr Gly Pro Val Arg Val	
130 135 140	
tcg ctg tat gac ctg gct tct gtg gac agc tgt gag gag aac tca gtg	480
Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn Ser Val	
145 150 155 160	
ctg gag atc att gcc ttt cat tgc aag agc ccg cac cga cac cga atg	528
Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His Arg Met	
165 170 175	
gtc gtt ttg gag ccc ctg aac aaa ctg ctg cag gcg aaa tgg gat ctg	576
Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp Asp Leu	
180 185 190	
ctc atc ccc aag ttc ttc tta aac ttc ctg tgt aat ctg atc tac atg	624
Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile Tyr Met	
195 200 205	
ttc atc ttc acc gct gtt gcc tac cat cag cct acc ctg aag aag cag	672
Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys Lys Gln	
210 215 220	
gcc gcc cct cac ctg aaa gcg gag gtt gga aac tcc atg ctg ctg acg	720
Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu Leu Thr	
225 230 235 240	
ggc cac atc ctt atc ctg cta ggg ggg atc tac ctc ctc gtg ggc cag	768
Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val Gly Gln	
245 250 255	
ctg tgg tac ttc tgg cgg cgc cac gtg ttc atc tgg atc tcg ttc ata	816
Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Ser Phe Ile	
260 265 270	
gac agc tac ttt gaa atc ctc ttc ctg ttc cag gcc ctg ctc aca gtg	864
Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu Thr Val	

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275	280	285	
gtg tcc cag gtg ctg tgt ttc ctg gcc atc gag tgg tac ctg ccc ctg			912
Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu Pro Leu			
290	295	300	
ctt gtg tct gcg ctg gtg ctg ggc tgg ctg aac ctg ctt tac tat aca			960
Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr Tyr Thr			
305	310	315	320
cgt ggc ttc cag cac aca ggc atc tac agt gtc atg atc cag aag aaa			1008
Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln Lys Lys			
	325	330	335
gcc atc tct gtc ctg gag atg gag aat ggc tat tgg tgg tgc agg aag			1056
Ala Ile Ser Val Leu Glu Met Glu Asn Gly Tyr Trp Trp Cys Arg Lys			
	340	345	350
aag cag cgg gca ggt gtg atg ctg acc gtt ggc act aag cca gat ggc			1104
Lys Gln Arg Ala Gly Val Met Leu Thr Val Gly Thr Lys Pro Asp Gly			
	355	360	365
agc ccg gat gag cgc tgg tgc ttc agg gtg gag gag gtg aac tgg gct			1152
Ser Pro Asp Glu Arg Trp Cys Phe Arg Val Glu Glu Val Asn Trp Ala			
	370	375	380
tca tgg gag cag acg ctg cct acg ctg tgt gag gac ccg tca ggg gca			1200
Ser Trp Glu Gln Thr Leu Pro Thr Leu Cys Glu Asp Pro Ser Gly Ala			
385	390	395	400
ggt gtc cct cga act ctc gag aac cct gtc ctg gct tcc cct ccc aag			1248
Gly Val Pro Arg Thr Leu Glu Asn Pro Val Leu Ala Ser Pro Pro Lys			
	405	410	415
gag gat gag gat ggt gcc tct gag gaa aac tat gtg ccc gtc cag ctc			1296
Glu Asp Glu Asp Gly Ala Ser Glu Glu Asn Tyr Val Pro Val Gln Leu			
	420	425	430
ctc cag tcc aac			1308
Leu Gln Ser Asn			
435			

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 <212> DNA
 <213> Rattus sp.

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<220>

<221> CDS

<222> (2)..(1663)

<400> 10

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    1             5             10             15

gat gtg gtg acc tac ctc ctg gag aac cca cac cag ccg gcc agc ctg 97
Asp Val Val Thr Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu
    20             25             30

gag gcc acc gac tcc ctg ggc aac aca gtc ctg cat gct ctg gta atg 145
Glu Ala Thr Asp Ser Leu Gly Asn Thr Val Leu His Ala Leu Val Met
    35             40             45

att gca gat aac tcg cct gag aac agt gcc ctg gtg atc cac atg tac 193
Ile Ala Asp Asn Ser Pro Glu Asn Ser Ala Leu Val Ile His Met Tyr
    50             55             60

gac gag ctt cta caa atg ggg gcg cgc ctc tgc ccc act gtg cag ctt 241
Asp Gly Leu Leu Gln Met Gly Ala Arg Leu Cys Pro Thr Val Gln Leu
    65             70             75             80

gag gaa atc tcc aac cac caa ggc ctc aca ccc ctg aaa cta gcc gcc 289
Glu Glu Ile Ser Asn His Gln Gly Leu Thr Pro Leu Lys Leu Ala Ala
    85             90             95

aag gaa ggc aaa atc gag att ttc agg cac att ctg cag cgg gaa ttc 337
Lys Glu Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe
    100            105            110

tca gga ccg tac cag ccc ctt tcc cga aag ttt act gag tgg tgt tac 385
Ser Gly Pro Tyr Gln Pro Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr
    115            120            125

ggt cct gtg cgg gta tcg ctg tac gac ctg tcc tct gtg gac agc tgg 433
Gly Pro Val Arg Val Ser Leu Tyr Asp Leu Ser Ser Val Asp Ser Trp
    130            135            140

gaa aag aac tcg gtg ctg gag atc atc gct ttt cat tgc aag agc ccg 481
Glu Lys Asn Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro
    145            150            155            160

aac cgg cac cgc atg gtg gtt tta gaa cca ctg aac aag ctt ctg cag 529
Asn Arg His Arg Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln
    165            170            175

gag aaa tgg gat cgg ctc gtc tca aga ttc ttc ttc aac ttc gcc tgc 577
Glu Lys Trp Asp Arg Leu Val Ser Arg Phe Phe Phe Asn Phe Ala Cys
    180            185            190

tac ttg gtc tac atg ttc atc ttc acc gtc gtt gcc tac cac cag cct 625
Tyr Leu Val Tyr Met Phe Ile Phe Thr Val Val Ala Tyr His Gln Pro
    195            200            205

tcc ctg gat cag cca gcc atc ccc tca tca aaa gcg act ttt ggg gaa 673

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Ser	Leu	Asp	Gln	Pro	Ala	Ile	Pro	Ser	Ser	Lys	Ala	Thr	Phe	Gly	Glu		
210						215					220						
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Ser	Met	Leu	Leu	Leu	Gly	His	Ile	Leu	Ile	Leu	Leu	Gly	Gly	Ile	Tyr		
225					230					235					240		
ctc	tta	ctg	ggc	cag	ctg	tgg	tac	ttt	tgg	cgg	cgg	cgc	ctg	ttt	atc	769	
Leu	Leu	Leu	Gly	Gln	Leu	Trp	Tyr	Phe	Trp	Arg	Arg	Arg	Leu	Phe	Ile		
				245					250					255			
tgg	atc	tca	ttc	atg	gac	agc	tac	ttt	gaa	atc	ctc	ttt	ctc	ctt	cag	817	
Trp	Ile	Ser	Phe	Met	Asp	Ser	Tyr	Phe	Glu	Ile	Leu	Phe	Leu	Leu	Gln		
			260					265					270				
gct	ctg	ctc	aca	gtg	ctg	tcc	cag	gtg	ctg	cgc	ttc	atg	gag	act	gaa	865	
Ala	Leu	Leu	Thr	Val	Leu	Ser	Gln	Val	Leu	Arg	Phe	Met	Glu	Thr	Glu		
			275				280					285					
tgg	tac	cta	ccc	ctg	cta	gtg	tta	tcc	cta	gtg	ctg	ggc	tgg	ctg	aac	913	
Trp	Tyr	Leu	Pro	Leu	Leu	Val	Leu	Ser	Leu	Val	Leu	Gly	Trp	Leu	Asn		
	290					295					300						
ctg	ctt	tac	tac	aca	cgg	ggc	ttt	cag	cac	aca	ggc	atc	tac	agt	gtc	961	
Leu	Leu	Tyr	Tyr	Thr	Arg	Gly	Phe	Gln	His	Thr	Gly	Ile	Tyr	Ser	Val		
305					310					315					320		
atg	atc	cag	aag	gtc	atc	ctt	cga	gac	ctg	ctc	cgt	ttc	ctg	ctg	gtc	1009	
Met	Ile	Gln	Lys	Val	Ile	Leu	Arg	Asp	Leu	Leu	Arg	Phe	Leu	Leu	Val		
				325					330					335			
tac	ctg	gtc	ttc	ctt	ttc	ggc	ttt	gct	gta	gcc	cta	gta	agc	ttg	agc	1057	
Tyr	Leu	Val	Phe	Leu	Phe	Gly	Phe	Ala	Val	Ala	Leu	Val	Ser	Leu	Ser		
			340					345						350			
aga	gag	gcc	cga	agt	ccc	aaa	gcc	cct	gaa	gat	aac	aac	tcc	aca	gtg	1105	
Arg	Glu	Ala	Arg	Ser	Pro	Lys	Ala	Pro	Glu	Asp	Asn	Asn	Ser	Thr	Val		
			355				360					365					
acg	gaa	cag	ccc	acg	gtg	ggc	cag	gag	gag	gag	cca	gct	cca	tat	cgg	1153	
Thr	Glu	Gln	Pro	Thr	Val	Gly	Gln	Glu	Glu	Glu	Pro	Ala	Pro	Tyr	Arg		
			370			375					380						
agc	att	ctg	gat	gcc	tcc	cta	gag	ctg	ttc	aag	ttc	acc	att	ggt	atg	1201	
Ser	Ile	Leu	Asp	Ala	Ser	Leu	Glu	Leu	Phe	Lys	Phe	Thr	Ile	Gly	Met		
385					390					395					400		
ggg	gag	ctg	gct	ttc	cag	gaa	cag	ctg	cgt	ttt	cgt	ggg	gtg	gtc	ctg	1249	
Gly	Glu	Leu	Ala	Phe	Gln	Glu	Gln	Leu	Arg	Phe	Arg	Gly	Val	Val	Leu		
				405					410					415			
ctg	ttg	ctg	ttg	gcc	tac	gtc	ctt	ctc	acc	tac	gtc	ctg	ctg	ctc	aac	1297	
Leu	Leu	Leu	Leu	Ala	Tyr	Val	Leu	Leu	Thr	Tyr	Val	Leu	Leu	Leu	Asn		
				420				425					430				
atg	ctc	att	gct	ctc	atg	agc	gaa	act	gtc	aac	cac	gtt	gct	gac	aac	1345	
Met	Leu	Ile	Ala	Leu	Met	Ser	Glu	Thr	Val	Asn	His	Val	Ala	Asp	Asn		
			435				440					445					

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agc tgg agc atc tgg aag ttg cag aaa gcc atc tct gtc ttg gag atg 1393
Ser Trp Ser Ile Trp Lys Leu Gln Lys Ala Ile Ser Val Leu Glu Met
450 455 460

gag aat ggt tac tgg tgg tgc cgg agg aag aaa cat cgt gaa ggg agg 1441
Glu Asn Gly Tyr Trp Trp Cys Arg Arg Lys Lys His Arg Glu Gly Arg
465 470 475 480

ctg ctg aaa gtc ggc acc agg ggg gat ggt acc cct gat gag cgc tgg 1489
Leu Leu Lys Val Gly Thr Arg Gly Asp Gly Thr Pro Asp Glu Arg Trp
485 490 495

tgc ttc agg gtg gag gaa gta aat tgg gct gct tgg gag aag act ctt 1537
Cys Phe Arg Val Glu Glu Val Asn Trp Ala Ala Trp Glu Lys Thr Leu
500 505 510

ccc acc tta tct gag gat cca tca ggg cca ggc atc act ggt aat aaa 1585
Pro Thr Leu Ser Glu Asp Pro Ser Gly Pro Gly Ile Thr Gly Asn Lys
515 520 525

aag aac cca acc tct aaa ccg ggg aag aac agt gcc tca gag gaa gac 1633
Lys Asn Pro Thr Ser Lys Pro Gly Lys Asn Ser Ala Ser Glu Glu Asp
530 535 540

cat ctg ccc ctt cag gtc ctc cag tcc ccc tgatggccca gatgcagcag 1683
His Leu Pro Leu Gln Val Leu Gln Ser Pro
545 550

caggctggca ggatggagta gggaaatcttc ccagccacac cagaggctac tgaatttttg 1743

tggaatatata aatatttttt ttgcataaaaa aaaaaaaaaa agggcggccg c 1794

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 <212> PRT
 <213> Rattus sp.

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20          25          30

Glu Ala Thr Asp Ser Leu Gly Asn Thr Val Leu His Ala Leu Val Met
35          40          45

Ile Ala Asp Asn Ser Pro Glu Asn Ser Ala Leu Val Ile His Met Tyr
50          55          60

Asp Gly Leu Leu Gln Met Gly Ala Arg Leu Cys Pro Thr Val Gln Leu
65          70          75          80

Glu Glu Ile Ser Asn His Gln Gly Leu Thr Pro Leu Lys Leu Ala Ala
85          90          95

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Lys Glu Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe
 100 105 110
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 Glu Lys Asn Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro
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 Glu Lys Trp Asp Arg Leu Val Ser Arg Phe Phe Phe Asn Phe Ala Cys
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 195 200 205
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 Arg Glu Ala Arg Ser Pro Lys Ala Pro Glu Asp Asn Asn Ser Thr Val
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 Thr Glu Gln Pro Thr Val Gly Gln Glu Glu Glu Pro Ala Pro Tyr Arg
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 Ser Ile Leu Asp Ala Ser Leu Glu Leu Phe Lys Phe Thr Ile Gly Met
 385 390 395 400
 Gly Glu Leu Ala Phe Gln Glu Gln Leu Arg Phe Arg Gly Val Val Leu
 405 410 415

- 33 -

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 att gca gat aac tcg cct gag aac agt gcc ctg gtg atc cac atg tac 192
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Gly Pro Val Arg Val Ser Leu Tyr Asp Leu Ser Ser Val Asp Ser Trp	
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Glu Lys Trp Asp Arg Leu Val Ser Arg Phe Phe Phe Asn Phe Ala Cys	
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- 35 -

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- 39 -

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 625 630

INTERNATIONAL SEARCH REPORT

Internat Application No

PCT/US 99/26701

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N5/10 C07K14/705 C07K16/28 G01N33/53
 C12Q1/68 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K G01N C12Q A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CATERINA M J ET AL: "THE CAPSAICIN RECEPTOR: A HEAT-ACTIVATED ION CHANNEL IN THE PAIN PATHWAY" NATURE, GB, MACMILLAN JOURNALS LTD. LONDON, vol. 389, 23 October 1997 (1997-10-23), pages 816-824, XP002075020 ISSN: 0028-0836 cited in the application abstract figure 5 page 821, left-hand column, paragraph 2	1-26
P, X	WO 99 09140 A (BRAKE ANTHONY ; JULIUS DAVID J (US); UNIV CALIFORNIA (US); CATERINA) 25 February 1999 (1999-02-25) abstract 100% identity in 1717 BP overlap between SEQ ID NO 3 of W09909140 and SEQ ID NO 10 --- -/-	1-26



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

28 April 2000

Date of mailing of the international search report

10/05/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Lejeune, R

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/ 26701

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 24-26 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Internat Application No

PCT/US 99/26701

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 37675 A (BRAKE ANTHONY J ;JULIUS DAVID J (US); UNIV CALIFORNIA (US); CATERI) 29 July 1999 (1999-07-29) abstract 99.7% identity in 2533 BP overlap between SEQ ID NO 33 of W09937675 and SEQ ID NO 1 100% identity in 2380 BP overlap between SEQ ID NO 35 of W09937675 and SEQ ID NO 4 100% identity in 1717 BP overlap between SEQ ID NO 3 of W09937675 and SEQ ID NO 10	1-26
P,X	WO 99 37765 A (SMITHKLINE BEECHAM PLC) 29 July 1999 (1999-07-29) abstract 99.9% identity in 2351 BP overlap between SEQ ID NO 1 and 5 of W09937765 and SEQ ID NO 4	1-26
P,X	EP 0 943 683 A (SMITHKLINE BEECHAM PLC) 22 September 1999 (1999-09-22) abstract 99.6% identity in 2818 BP overlap between SEQ ID NO 1 of EP943683 and SEQ ID NO 1	1-26
P,X	EP 0 953 638 A (SYNTHELABO) 3 November 1999 (1999-11-03) abstract 99.8% identity in 2786 BP overlap between SEQ ID NO 1 of EP953638 and SEQ ID NO 4	1-26
P,X	CATERINA ET AL: "A capsaicin-receptor homologue with a high threshold for noxious heat" NATURE,GB,MACMILLAN JOURNALS LTD. LONDON, vol. 398, no. 398, 1 April 1999 (1999-04-01), pages 436-441-441, XP002105951 ISSN: 0028-0836 the whole document 99.8% identity in 2401 BP overlap between the sequence of hVRL and SEQ ID NO 4 100% identity in 1752 BP overlap between the sequence of rVRL and SEQ ID NO 10	1-26
X	WO 98 39448 A (HUMAN GENOME SCI INC) 11 September 1998 (1998-09-11) abstract page 112 page 163 99.3% identity in 1947 BP overlap between SEQ ID NO 191 of W09839448 and SEQ ID NO 4 98.1% identity in 1709 BP overlap between SEQ ID NO 307 of W09839448 and SEQ ID NO 4	1-11

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 99/26701

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9909140	A	25-02-1999	AU 9115698 A AU 2466799 A WO 9937675 A	08-03-1999 09-08-1999 29-07-1999
WO 9937675	A	29-07-1999	AU 2466799 A AU 9115698 A WO 9909140 A	09-08-1999 08-03-1999 25-02-1999
WO 9937765	A	29-07-1999	NONE	
EP 0943683	A	22-09-1999	JP 11279196 A	12-10-1999
EP 0953638	A	03-11-1999	AU 2932799 A WO 9946377 A	27-09-1999 16-09-1999
WO 9839448	A		NONE	



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/12, 5/10, C07K 14/705, 16/28, G01N 33/53, C12Q 1/68, A61P 25/00	A1	(11) International Publication Number: WO 00/29577 (43) International Publication Date: 25 May 2000 (25.05.00)												
(21) International Application Number: PCT/US99/26701 (22) International Filing Date: 12 November 1999 (12.11.99) (30) Priority Data: <table border="0"><tr><td>60/108,322</td><td>13 November 1998 (13.11.98)</td><td>US</td></tr><tr><td>60/114,078</td><td>28 December 1998 (28.12.98)</td><td>US</td></tr><tr><td>09/258,633</td><td>26 February 1999 (26.02.99)</td><td>US</td></tr><tr><td>09/421,134</td><td>19 October 1999 (19.10.99)</td><td>US</td></tr></table> (71) Applicant: MILLENNIUM PHARMACEUTICALS, INC. [US/US]; 75 Sidney Street, Cambridge, MA 02139 (US). (72) Inventor: CURTIS, Rory, A., J.; 31 Constitution Drive, Southborough, MA 01772 (US). (74) Agents: MANDRAGOURAS, Amy, E.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US) et al.		60/108,322	13 November 1998 (13.11.98)	US	60/114,078	28 December 1998 (28.12.98)	US	09/258,633	26 February 1999 (26.02.99)	US	09/421,134	19 October 1999 (19.10.99)	US	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
60/108,322	13 November 1998 (13.11.98)	US												
60/114,078	28 December 1998 (28.12.98)	US												
09/258,633	26 February 1999 (26.02.99)	US												
09/421,134	19 October 1999 (19.10.99)	US												
(54) Title: NOVEL MEMBERS OF THE CAPSAICIN/VANILLOID RECEPTOR FAMILY OF PROTEINS AND USES THEREOF														
(57) Abstract <p>The invention provides isolated nucleic acids molecules, designated hVR-1, hVR-2, and rVR-2 nucleic acid molecules, which encode novel members of the Capsaicin/Vanilloid receptor family. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing hVR-1, hVR-2, and rVR-2 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which an hVR-1, hVR-2, and rVR-2 gene has been introduced or disrupted. The invention still further provides isolated hVR-1, hVR-2, and rVR-2 proteins, fusion proteins, antigenic peptides and anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.</p>														

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NOVEL MEMBERS OF THE CAPSAICIN/VANILLOID RECEPTOR FAMILY OF PROTEINS AND USES THEREOF

Background of the Invention

5 Pain is initiated when the peripheral terminals of a subgroup of sensory neurons are activated by noxious chemical, mechanical or thermal stimuli. These neurons, called nociceptors, transmit information regarding tissue damage to pain-processing centres in the spinal chord and brain (Fields, H.L. *Pain*, McGraw-Hill, New York, 1987). Nociceptors are characterized in part, by their sensitivity to capsaicin, a vanilloid-
10 containing compound, and a natural product of capsicum peppers that is the active ingredient of many "hot" and spicy foods. In mammals, exposure of nociceptor terminals to capsaicin leads initially to excitation of the neuron and the consequent perception of pain and local release of inflammatory mediators. With prolonged exposure, nociceptor terminals become insensitive to capsaicin, as well as to other
15 noxious stimuli (Szolcsanyi, J. in *Capsaicin in the Study of Pain* (ed. Wood, J.) 1-26 (Academic, London, 1993). This latter phenomenon of nociceptor desensitization underlies the seemingly paradoxical use of capsaicin as an analgesic agent in the treatment of painful disorders ranging from viral and diabetic neuropathies to rheumatoid arthritis (Campbell, E. in *Capsaicin and the Study of Pain* (ed. Wood, J.)
20 255-272 (Academic, London, 1993); Szallasi, A. *et al.* (1996) *Pain* 68, 195-208). Some of this decreased sensitivity to noxious stimuli may result from reversible changes in the nociceptor, but the long-term loss of responsiveness can be explained by death of the nociceptor or destruction of its peripheral terminals following exposure to capsaicin (Jancso, G. *et al.* (1977) *Nature* 270, 741-743).

25 The cellular specificity of capsaicin action and its ability to evoke the sensation of burning pain have led to speculation that the target of capsaicin action plays an important physiological role in the detection of painful stimuli. Indeed, capsaicin may elicit the perception of pain by mimicking the actions of a physiological stimulus or an endogenous ligand produced during tissue injury (James, I.F., Kinkina, N.N. & Wood, J.N. in *Capsaicin in the Study of Pain* (ed. Wood, J.N.) 83-104 (Academic, London,
30 1993).

Caterina M.J. *et al.* have recently determined the molecular basis underlying this phenomenon by characterizing a functional cDNA that encodes a vanilloid receptor (VR-1) in rat sensory ganglia (Caterina M. J. *et al.*, (1997) *Nature* 389:816-824). VR-1 is a vanilloid-gated, nonselective cation channel that resembles members of the transient receptor potential (TRP) channel family, first identified as components of the *Drosophila* phototransduction pathway (Montell *et al.* (1989) *Neuron* 2:1313-1323).

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Summary of the Invention

The present invention is based, at least in part, on the discovery of novel members of the Capsaicin/Vanilloid family of receptors. Described herein is the isolation of the human orthologue of rat VR-1 (rVR-1), referred to herein as hVR-1, as well as another previously unknown member of the VR family of receptors, referred herein as VR-2, and specifically as human VR-2 (hVR-2, including an alternate form which contains a deletion) and rat VR-2 (rVR-2) nucleic acid and protein molecules. The hVR-1, hVR-2, and rVR-2 molecules of the present invention are useful as targets for developing modulating agents to regulate a variety of cellular processes, *e.g.*, cellular processes involved in pain. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding hVR-1, hVR-2, and rVR-2 proteins and fragments thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of hVR-1, hVR-2, and rVR-2-encoding nucleic acids.

In one embodiment, an hVR-1, hVR-2, or rVR-2 nucleic acid molecule of the invention is at least 60%, 65%, 70%, 75%, 80%, 83%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 or a complement thereof.

In another embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a complement thereof. In another embodiment, the nucleic acid molecule includes at least 10, 15, 20, or more contiguous nucleotides of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

In another embodiment, an hVR-1, hVR-2, and rVR-2 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, 5, 8, or 11. In one embodiment, an hVR-1, hVR-2, and rVR-2 nucleic acid molecule includes a
5 nucleotide sequence encoding a protein having an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 95%, 98% or more identical to the entire length of the amino acid sequence of SEQ ID NO:2, 5, 8, or 11.

Another embodiment of the invention features nucleic acid molecules, preferably hVR-1, hVR-2, and rVR-2 nucleic acid molecules, which specifically detect hVR-1,
10 hVR-2, and rVR-2 nucleic acid molecules relative to nucleic acid molecules encoding non-hVR-1, non-hVR-2, and non-hVR-2 proteins. For example, in one embodiment, such a nucleic acid molecule is at least 100-150, 1150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-700, 700-800, 800-900, 900-1000, 1088, or more nucleotides in length and hybridizes under stringent conditions to a
15 nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-17, 3696-3863, or 3901-3909 of SEQ ID NO:1. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-17, 3696-3863, or
20 3901-3909 of SEQ ID NO:1. In yet other preferred embodiments, the nucleic acid molecules consist of nucleotides 1-17, 3696-3863, or 3901-3909 of SEQ ID NO:1. In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1944-2003 of SEQ ID NO:4. In other preferred embodiments, the nucleic acid molecules comprise
25 nucleotides 1944-2003 of SEQ ID NO:4. In yet other preferred embodiments, the nucleic acid molecules consist of nucleotides 1944-2003 of SEQ ID NO:4.

In other embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule
30 consisting of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 under stringent conditions and is encoded by the same locus as hVR-1, hVR-2 or rVR-2.

Another embodiment of the invention provides a nucleic acid molecule that encodes a naturally occurring orthologue of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule consisting of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 under stringent
5 conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to an hVR-1, hVR-2, and rVR-2 nucleic acid molecule, *e.g.*, the coding strand of an hVR-1, hVR-2, and rVR-2 nucleic acid molecule.

Since the hVR2 (the alternate form) and rVR2 sequences represent fragments of
10 the entire coding regions of these genes, another embodiment of the invention provides the complete gene sequences. A skilled artisan can readily isolate such molecule using the sequences disclosed herein.

Another aspect of the invention provides a vector comprising an hVR-1, an hVR-2, or a rVR-2 nucleic acid molecule. In certain embodiments, the vector is a
15 recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. In yet another embodiment, the invention provides a host cell containing a nucleic acid molecule of the invention. The invention also provides a method for producing a protein, preferably an hVR-1, hVR-2, and rVR-2 protein, by culturing in a suitable medium, a host cell, *e.g.*, a mammalian host cell such
20 as a non-human mammalian cell. of the invention containing a recombinant expression vector, such that the protein is produced.

Another aspect of this invention features isolated or recombinant hVR-1, hVR-2, and rVR-2 proteins and polypeptides. In one embodiment, the isolated protein, preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane
25 domain. In another embodiment, the isolated protein, preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane domain and at least one proline rich domain. In yet another embodiment, the isolated protein, preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane domain, at least one proline rich domain, and at least one ankyrin repeat domain. In yet another embodiment, the protein,
30 preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane domain, at least one proline rich domain, and at least one ankyrin repeat domain and has

an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 95%, 98% or more homologous to the amino acid sequence of SEQ ID NO:2, 5, 8, or 11. In another embodiment, the protein, preferably an hVR-1, hVR-2, or rVR-2 protein, includes at least one transmembrane domain, at least one proline rich domain, and at least one ankyrin repeat domain and plays a role in the development and regulation of pain. In yet another embodiment, the protein, preferably an hVR-1, hVR-2, and rVR-2 protein, includes at least one transmembrane domain, at least one proline rich domain, and at least one ankyrin repeat domain and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

In another embodiment, the invention features fragments of the protein having the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the fragment comprises at least 15, 30, 40, 50, 60, 70, 80, 90, or 100 amino acids (*e.g.*, contiguous amino acids).

In another embodiment, the invention features an isolated protein, preferably an hVR-1, hVR-2, and rVR-2 protein, which is encoded by a nucleic acid molecule consisting of a nucleotide sequence at least about 60%, 65%, 70%, 75%, 80%, 83%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a complement thereof. This invention further features an isolated protein, preferably an hVR-1, hVR-2, or rVR-2 protein, which is encoded by a nucleic acid molecule consisting of a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule consisting of the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a complement thereof.

The proteins of the present invention or portions thereof, *e.g.*, biologically active portions thereof, can be operatively linked to a non-hVR-1, non-hVR-2, or non-rVR-2 polypeptide (*e.g.*, heterologous amino acid sequences) to form fusion proteins. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention, preferably hVR-1, hVR-2, and rVR-2 proteins. In addition, the hVR-1, hVR-2, and rVR-2 proteins or biologically active

portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of an hVR-1, hVR-2, and rVR-2 nucleic acid molecule, protein or polypeptide in a biological sample by contacting the biological sample with an agent capable of
5 detecting an hVR-1, hVR-2, and rVR-2 nucleic acid molecule, protein or polypeptide such that the presence of an hVR-1, hVR-2, and rVR-2 nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the
10 presence of hVR-1, hVR-2, and rVR-2 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of hVR-1, hVR-2, and rVR-2 activity such that the presence of hVR-1, hVR-2, and rVR-2 activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating hVR-1, hVR-
15 2, and rVR-2 activity comprising contacting a cell capable of expressing hVR-1, hVR-2, and rVR-2 with an agent that modulates hVR-1, hVR-2, and rVR-2 activity such that hVR-1, hVR-2, and rVR-2 activity in the cell is modulated. In one embodiment, the agent inhibits hVR-1, hVR-2, and rVR-2 activity. In another embodiment, the agent stimulates hVR-1, hVR-2, and rVR-2 activity. In one embodiment, the agent is an
20 antibody that specifically binds to an hVR-1, hVR-2, and rVR-2 protein. In another embodiment, the agent modulates expression of hVR-1, hVR-2, and rVR-2 by modulating transcription of an hVR-1, hVR-2, and rVR-2 gene or translation of an hVR-1, hVR-2, and rVR-2 mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of an hVR-
25 1, hVR-2, and rVR-2 mRNA or an hVR-1, hVR-2, and rVR-2 gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant hVR-1, hVR-2, and rVR-2 protein or nucleic acid expression or activity by administering an agent which is an hVR-1, hVR-2, and rVR-2 modulator to the subject. In one embodiment, the hVR-1, hVR-2, and rVR-2
30 modulator is an hVR-1, hVR-2, and rVR-2 protein. In another embodiment the hVR-1, hVR-2, and rVR-2 modulator is an hVR-1, hVR-2, and rVR-2 nucleic acid molecule. In

yet another embodiment, the hVR-1, hVR-2, and rVR-2 modulator is a peptide, peptidomimetic, or other small molecule. In a further embodiment, the disorder characterized by aberrant hVR-1, hVR-2, and rVR-2 protein or nucleic acid expression is a pain disorder, *e.g.*, hyperalgesia.

5 The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding an hVR-1, hVR-2, and rVR-2 protein; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of an hVR-1, hVR-2, and rVR-2 protein, wherein a wild-type form of the gene encodes a protein
10 with an hVR-1, hVR-2, and rVR-2 activity (as described herein).

 In another aspect the invention provides a method for identifying a compound that binds to or modulates the activity of an hVR-1, hVR-2, and rVR-2 protein, by providing an indicator composition comprising an hVR-1, hVR-2, and rVR-2 protein having hVR-1, hVR-2, and rVR-2 activity, contacting the indicator composition with a
15 test compound, and determining the effect of the test compound on hVR-1, hVR-2, and rVR-2 activity in the indicator composition to identify a compound that modulates the activity of an hVR-1, hVR-2, and rVR-2 protein.

 Other features and advantages of the invention will be apparent from the following detailed description and claims.

20

Brief Description of the Drawings

 Figure 1 depicts the full length cDNA sequence and predicted amino acid sequence of human VR-1 (hVR-1). The nucleotide sequence corresponds to nucleic acids 1 to 3909 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids
25 1 to 839 of SEQ ID NO:2. The coding region without the 5' and 3' untranslated regions of the human VR-1 (hVR-1) gene is shown in SEQ ID NO:3.

 Figure 2 depicts the full length cDNA sequence and predicted amino acid sequence of human VR-2 (hVR-2). The nucleotide sequence corresponds to nucleic acids 1 to 2809 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids
30 1 to 764 of SEQ ID NO:5. The coding region without the 5' and 3' untranslated regions of the human VR-2 (hVR-2) gene is shown in SEQ ID NO:6.

Figure 3 depicts the partial cDNA sequence and partial predicted amino acid sequence of an alternate form of human VR-2 (hVR-2). The nucleotide sequence corresponds to nucleic acids 1 to 1489 of SEQ ID NO:7. The amino acid sequence corresponds to amino acids 1 to 436 of SEQ ID NO:8. The coding region without the 5' and 3' untranslated regions of the alternate form of human VR-2 (hVR-2) gene is shown in SEQ ID NO:9.

Figure 4 depicts the partial cDNA sequence and partial predicted amino acid sequence of rat VR-2 (rVR-2). The nucleotide sequence corresponds to nucleic acids 1 to 1794 of SEQ ID NO:10. The amino acid sequence corresponds to amino acids 1 to 554 of SEQ ID NO:11. The coding region without the 5' and 3' untranslated regions of the rat VR-2 (rVR-2) gene is shown in SEQ ID NO:12.

Figure 5 depicts an alignment of the hVR-1 protein (SEQ ID NO:2) with the human VR-2 protein (SEQ ID NO:5) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4.

Figure 6 depicts an alignment of the hVR-1 nucleotide sequence (SEQ ID NO:1) with the human VR-2 nucleotide sequence (SEQ ID NO:4) using the GAP program in the GCG software package (nwsgapdna matrix) and a gap weight of 50 and a length weight of 3.

Figure 7 depicts an alignment of the hVR-2 protein (SEQ ID NO:5) with the rat VR-2 protein (SEQ ID NO:11) using the CLUSTAL W (1.74) multiple sequence alignment program.

Figure 8 depicts an alignment of the hVR-2 protein (SEQ ID NO:5) with the rat VR-2 protein (SEQ ID NO:11) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4.

Figure 9 depicts an alignment of the hVR-1 nucleotide sequence (SEQ ID NO:1) with the rat VR-1 nucleotide sequence (Accession Number:AF029310) using the GAP program in the GCG software package (nwsgapdna matrix) and a gap weight of 50 and a length weight of 3.

Figure 10 depicts an alignment of the hVR-1 protein (SEQ ID NO:2) with the rat VR-1 protein (Accession Number:AF029310) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4.

Figure 11 depicts an alignment of the hVR-2 protein (SEQ ID NO:5) with the human VR-2 protein (alternate form) (SEQ ID NO:8) using the CLUSTAL W (1.74) multiple sequence alignment program.

Figure 12 depicts a structural, hydrophobicity, and antigenicity analysis of the hVR-1 protein.

Figure 13 depicts the results of a search using the amino acid sequence of the hVR-1 protein against the HMM database.

Figure 14 depicts a structural, hydrophobicity, and antigenicity analysis of the hVR-2 protein.

Figure 15 depicts the results of a search using the amino acid sequence of the hVR-2 protein against the HMM database.

Figure 16 depicts the predicted full length amino acid sequence of the human VR-2 protein (alternate form) (SEQ ID NO:20).

Figure 17 depicts an alignment of the hVR-2 protein (SEQ ID NO:5) with the predicted full length human VR-2 protein (alternate form) (SEQ ID NO:20) using the CLUSTAL W (1.74) multiple sequence alignment program.

Detailed Description of the Invention

The present invention is based, at least in part, on the discovery of nucleic acid and amino acid molecules which are novel members of the Capsaicin/Vanilloid family of receptors. Described herein is the isolation of the human orthologue of rat VR-1 (rVR-1), referred to herein as hVR-1, as well as another previously unknown member of the VR family of receptors, referred to herein as VR-2, and specifically as human VR-2 (hVR-2) and rat VR-2 (rVR-2) nucleic acid and protein molecules. The hVR-1, hVR-2, and rVR-2 molecules were identified based on their sequence similarity to the known rat vanilloid receptor (VR-1). VR-1 is a vanilloid gated, non-selective cation channel which resembles members of the transient receptor potential (TRP) ion channel family (described in Montell *et al.* (1989) *Neuron* 2:1313-1323) that mediate the influx of extracellular calcium in response to depletion of intracellular calcium stores. The rat VR-1 cDNA contains an open reading frame of 2514 nucleotides that encodes a protein of 838 amino acids. Hydrophilicity analysis has indicated that rat VR-1 contains six

transmembrane domains (predicted to be mostly α -helices) with an additional short hydrophobic stretch between transmembrane regions 5 and 6. The amino terminal hydrophilic segment contains a relatively proline rich region followed by three ankyrin repeat domains. The rat VR-1 is expressed in small diameter neurons within sensory ganglia. The present hVR-1 sequence is the human orthologue of rVR-1. As described in further detail *infra*, the human VR-1 is expressed in nodose, trigeminal sensory neurons, as well as in some, but not all, small dorsal root ganglion (DRG) neurons and in a few medium sized DRG neurons.

The hVR-1, hVR-2, and rVR-2 molecules of the present invention play a role in pain signaling mechanisms. As used herein, the term "pain signaling mechanisms" includes the cellular mechanisms involved in the development and regulation of pain, *e.g.*, pain elicited by noxious chemical, mechanical, or thermal stimuli, in a subject, *e.g.*, a mammal such as a human. In mammals, the initial detection of noxious chemical, mechanical, or thermal stimuli, a process referred to as "nociception", occurs predominantly at the peripheral terminals of specialized, small diameter primary afferent neurons, called polymodal nociceptors. These afferent neurons transmit the information to the central nervous system, evoking a perception of pain or discomfort and initiating appropriate protective reflexes. Capsaicin/Vanilloid receptors, *e.g.*, the hVR-1, hVR-2, and rVR-2 molecules of the present invention, present on these afferent neurons, are involved in detecting these noxious chemical, mechanical, or thermal stimuli and transducing this information into membrane depolarization events. Thus, the hVR-1, hVR-2, and rVR-2 molecules by participating in pain signaling mechanisms, can modulate pain elicitation and provide novel diagnostic targets and therapeutic agents to control pain.

The hVR-1, hVR-2, and rVR-2 molecules provide novel diagnostic targets and therapeutic agents to control pain in a variety of disorders, diseases, or conditions which are characterized by a deregulated, *e.g.*, upregulated or downregulated, pain response. For example, the hVR-1, hVR-2, and rVR-2 molecules provide novel diagnostic targets and therapeutic agents to control the exaggerated pain response elicited during various forms of tissue injury, *e.g.*, inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L. (1987) *Pain*, New York:McGraw-

Hill). Moreover, the hVR-1, hVR-2, and rVR-2 molecules provide novel diagnostic targets and therapeutic agents to control pain associated with musculoskeletal disorders, *e.g.*, joint pain; tooth pain; headaches; pain associated with surgery, or neuropathic pain.

As the hVR-1 gene maps to a region of human chromosome 17 between WI-
5 5436 (7.7cR) and WI-6584 (18.9cR) (Example 6), which has been associated with myasthenia gravis, Smith-Magenis syndrome, CORD5, Cone-rod dysrrophy, and breast cancer, the hVR-1 molecule may provide novel diagnostic targets and therapeutic agents to treat, diagnose, or prognose these disorders or other disorders linked to this chromosomal region. Similarly, as the hVR-2 gene maps to a region of human
10 chromosome 17 between AFMA043ZB5 (23.3 cR) and D17S721 (29.3cR) (Example 6) which has been associated with myasthenia gravis, Smith-Magenis syndrome, CORD5, Cone-rod dysrrophy, choroidal dystrophy, central areolar, and retinal cone dystrophy, the hVR-2 molecule may provide novel diagnostic targets and therapeutic agents to treat, diagnose, or prognose these disorders or other disorders linked to this chromosomal
15 region.

The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-
20 naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics.

For example, the family of hVR-1, hVR-2, and rVR-2 proteins comprise at least
25 one, and preferably six "transmembrane domains." As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 20, 25, 30, 35, 40, or 45 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in
30 hydrophobic residues, and typically have a helical structure. In a embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acid residues of a

transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, Zagotta W.N. et al, (1996) *Annual Rev. Neurosci.* 19: 235-63, the contents of which are incorporated herein by reference. Amino acid residues 434-455, 480-495, (509-531; based on
5 homology to the rat VR-1) or 514-531, (543-569; based on homology to the rat VR-1) or 538-555, (577-596; based on homology to the rat VR-1) or 580-599, and (656-683; based on homology to the rat VR-1) or 658-682 of hVR-1 (SEQ ID NO:2) and amino acid residues 391-410, 431-448, 459-476, 486-508, 538-556, and 621-645 of hVR-2 (SEQ ID NO:5) comprise transmembrane domains.

10 In another embodiment, an hVR-1, hVR-2, and rVR-2 of the present invention is identified based on the presence of a "proline rich domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "proline rich domain" includes an amino acid sequence of about 4-6 amino acid residues in length having the general sequence X-Pro-X-X-Pro-X (where X can be any amino acid). Proline rich
15 domains are usually located in a helical structure and bind through hydrophobic interactions to SH3 domains. SH3 domains recognize proline rich domains in both forward and reverse orientations. Proline rich domains are described in, for example, Sattler M. *et al.* (1998) *Leukemia* 12:637-644, the contents of which are incorporated herein by reference.

20 In another embodiment, an hVR-1, hVR-2, and rVR-2 of the present invention is identified based on the presence of an "ankyrin repeat domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "ankyrin repeat domain" includes a protein domain having an amino acid sequence of about 30-50 amino acid residues and having a bit score for the alignment of the sequence to the ankyrin repeat
25 domain (HMM) of at least 6. Preferably, an ankyrin repeat domain includes at least about 30-45, more preferably about 30-40 amino acid residues, or about 30-35 amino acids and has a bit score for the alignment of the sequence to the ankyrin repeat domain (HMM) of at least 3-10, more preferably 10-30, more preferably 30-50, even more preferably 50-75, 75-100, 100-200 or greater. The ankyrin repeat domain HMM has
30 been assigned the PFAM Accession PF00023 (<http://genome.wustl.edu/Pfam/.html>). Ankyrin repeats are involved in protein-protein interactions and are described in, for

example, Ketchum K.A *et al.* (1996) *FEBS Letters* 378:19-26, the contents of which are incorporated herein by reference.

To identify the presence of an ankyrin repeat domain in an hVR-1, hVR-2, and rVR-2 protein and make the determination that a protein of interest has a particular
5 profile, the amino acid sequence of the protein is searched against a database of HMMs (*e.g.*, the Pfam database, release 2.1) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28(3)405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.* (1990) *Meth.*
10 *Enzymol.* 183:146-159; Gribskov *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.* (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.* (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference. A search was performed against the HMM database resulting in the identification of three ankyrin repeat domains in the amino acid sequence of SEQ ID NO:2 (at about residues 201-233,
15 248-283, and 333-361) and SEQ ID NO:5 (at about residues 162-194, 208-243, and 293-328). The results of the searches are set forth in Figures 13 and 15.

Isolated proteins of the present invention, preferably hVR-1, hVR-2, and rVR-2 proteins, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2, 5, 8, or 11 or are encoded by a nucleotide sequence sufficiently identical to
20 SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural
25 domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% identity, preferably 60% identity, more preferably 70%-80%, and even more preferably 90-95% identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently
30 identical. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%,

or 50%, preferably 60%, more preferably 70-80%, or 90-95% identity and share a common functional activity are defined herein as sufficiently identical.

As used interchangeably herein, an "hVR-1, hVR-2, and rVR-2 activity", "biological activity of hVR-1, hVR-2, and rVR-2" or "functional activity of hVR-1, hVR-2, and rVR-2",
5 refers to an activity exerted by an hVR-1, hVR-2, and rVR-2 protein, polypeptide or nucleic acid molecule on an hVR-1, hVR-2, and rVR-2 responsive cell or on an hVR-1, hVR-2, and rVR-2 protein substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, an hVR-1, hVR-2, and rVR-2 activity is a direct activity, such as an association with an hVR-1, hVR-2, and rVR-2-target molecule. As used herein, a "target
10 molecule" or "binding partner" is a molecule with which an hVR-1, hVR-2, and rVR-2 protein binds or interacts in nature, such that hVR-1, hVR-2, and rVR-2-mediated function is achieved. An hVR-1, hVR-2, and rVR-2 target molecule can be a non-hVR-1, non-hVR-2, and non-rVR-2 molecule or an hVR-1, hVR-2, and rVR-2 protein or polypeptide of the present invention. In an exemplary embodiment, an hVR-1, hVR-2, and rVR-2 target
15 molecule is an hVR-1, hVR-2, and rVR-2 ligand, *e.g.*, capsaicin. Alternatively, an hVR-1, hVR-2, and rVR-2 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the hVR-1, hVR-2, and rVR-2 protein with an hVR-1, hVR-2, and rVR-2 ligand.

Accordingly, another embodiment of the invention features isolated hVR-1,
20 hVR-2, and rVR-2 proteins and polypeptides having an hVR-1, hVR-2, and rVR-2 activity. Other proteins of the invention are hVR-1, hVR-2, and rVR-2 proteins having at least one, and preferably six, transmembrane domains and, preferably, an hVR-1, hVR-2, and rVR-2 activity. Yet other proteins of the invention are hVR-1, hVR-2, and rVR-2 proteins having at least one transmembrane domain, at least one proline rich
25 domain and, preferably, an hVR-1, hVR-2, and rVR-2 activity. Other proteins of the invention are hVR-1, hVR-2, and rVR-2 proteins having at least one transmembrane domain, at least one proline rich domain, at least one ankyrin repeat domain and, preferably, an hVR-1, hVR-2, and rVR-2 activity. Additional proteins of the invention have at least one transmembrane domain, at least one proline rich domain, at least one
30 ankyrin repeat domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a

nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

The nucleotide sequence of the full length hVR-1 cDNA and the predicted amino acid sequence of the hVR-1 polypeptide are shown in Figure 1 and in SEQ ID
5 NOs:1 and 2, respectively.

The nucleotide sequence of the full length hVR-2 cDNA and the predicted amino acid sequence of the hVR-2 polypeptide are shown in Figure 2 and in SEQ ID NOs:4 and 5, respectively.

The nucleotide sequence of the partial hVR-2 (alternate form) cDNA and the
10 predicted amino acid sequence of the hVR-2 (alternate form) polypeptide are shown in Figure 3 and in SEQ ID NOs:7 and 8, respectively.

The nucleotide sequence of the partial rVR-2 cDNA and the predicted amino acid sequence of the rVR-2 polypeptide are shown in Figure 4 and in SEQ ID NOs:10 and 11, respectively.

15 Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that
20 encode hVR-1, hVR-2, and rVR-2 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify hVR-1, hVR-2, and rVR-2-encoding nucleic acid molecules (*e.g.*, hVR-1, hVR-2, and rVR-2 mRNA) and fragments for use as PCR primers for the amplification or mutation of hVR-1, hVR-2, and rVR-2 nucleic acid molecules. As used herein, the term "nucleic acid
25 molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which
30 are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated"

includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated hVR-1, hVR-2, and rVR-2 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, as a hybridization probe, hVR-1, hVR-2, and rVR-2 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to hVR-1, hVR-2, and rVR-2 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In one embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the full length hVR-1 encoding cDNA.

In another embodiment, an isolated nucleic acid molecule of the invention
5 comprises the nucleotide sequence shown in SEQ ID NO:4. The sequence of SEQ ID NO:4 corresponds to the full length hVR-2 encoding cDNA.

In another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:7. The sequence of SEQ ID NO:7 corresponds to a fragment of the hVR-2 (alternate form) encoding cDNA.

10 In another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:10. The sequence of SEQ ID NO:10 corresponds to a fragment of the rVR-2 cDNA.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence
15 shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6,
20 7, 9, 10, or 12 thereby forming a stable duplex.

In still another embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 60%, 65%, 70%, 75%, 80%, 83%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to the entire length of the nucleotide sequence shown in
25 SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of an hVR-1, hVR-2, and rVR-2 protein, *e.g.*, a biologically active portion of an hVR-1, hVR-
30 2, and rVR-2 protein. The nucleotide sequence determined from the cloning of the hVR-1, hVR-2, and rVR-2 gene allows for the generation of probes and primers

designed for use in identifying and/or cloning other hVR-1, hVR-2, and rVR-2 family members, as well as hVR-1, hVR-2, and rVR-2 homologues from other species. The probe/primer typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes
5 under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, 75, or 100 consecutive nucleotides of a sense sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, of an anti-sense sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. In an exemplary embodiment, a nucleic acid
10 molecule of the present invention comprises a nucleotide sequence which is greater than 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1088, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

15 Probes based on the hVR-1, hVR-2, and rVR-2 nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for
20 identifying cells or tissue which misexpress an hVR-1, hVR-2, and rVR-2 protein, such as by measuring a level of an hVR-1, hVR-2, and rVR-2-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting hVR-1, hVR-2, and rVR-2 mRNA levels or determining whether a genomic hVR-1, hVR-2, and rVR-2 gene has been mutated or deleted.

25 A nucleic acid fragment encoding a "biologically active portion of an hVR-1, hVR-2, and rVR-2 protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, which encodes a polypeptide having an hVR-1, hVR-2, and rVR-2 biological activity (the biological activities of the hVR-1, hVR-2, and rVR-2 proteins are described herein), expressing the encoded portion of the
30 hVR-1, hVR-2, and rVR-2 protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the hVR-1, hVR-2, and rVR-2 protein.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, due to degeneracy of the genetic code and thus encode the same hVR-1, hVR-2, and rVR-2 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, 5, 8, or 11.

In addition to the hVR-1, hVR-2, and rVR-2 nucleotide sequences shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the hVR-1, hVR-2, and rVR-2 proteins may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the hVR-1, hVR-2, and rVR-2 genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding an hVR-1, hVR-2, and rVR-2 protein, preferably a mammalian hVR-1, hVR-2, and rVR-2 protein, and can further include non-coding regulatory sequences, and introns.

Allelic variants of hVR-1, hVR-2, and rVR-2 include both functional and non-functional hVR-1, hVR-2, and rVR-2 proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the hVR-1, hVR-2, and rVR-2 protein that maintain the ability to bind an hVR-1, hVR-2, and rVR-2 ligand and/or modulate a pain signaling mechanism. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, 5, 8, or 11, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the hVR-1, hVR-2, and rVR-2 protein that do not have the ability to either bind an hVR-1, hVR-2, and rVR-2 ligand and/or modulate a pain signaling mechanism. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID

NO:2, 5, 8, or 11, or a substitution, insertion or deletion in critical residues or critical regions.

The present invention further provides non-human orthologues of the hVR-2 and rVR-2 protein. Orthologues of the hVR-2 and rVR-2 protein are proteins that are
5 isolated from non-human and non-rat organisms and possess the same hVR-2 and rVR-2 ligand binding and/or modulation of pain signaling mechanism capabilities of the hVR-2 and rVR-2 proteins. Orthologues of the hVR-2 and rVR-2 proteins can readily be identified as comprising an amino acid sequence that is substantially homologous to SEQ ID NO: 4, 6, 8 or 10.

10 Moreover, nucleic acid molecules encoding other hVR-1, hVR-2, and rVR-2 family members and, thus, which have a nucleotide sequence which differs from the hVR-1, hVR-2, and rVR-2 sequences of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, are intended to be within the scope of the invention. For example, another hVR-1, hVR-2, and rVR-2 cDNA can be identified based on the nucleotide sequence of hVR-1, hVR-2,
15 and rVR-2. Moreover, nucleic acid molecules encoding VR-2 proteins from different species, and which, thus, have a nucleotide sequence which differs from the hVR-2 and rVR-2 sequences of SEQ ID NO:4, 6, 8, or 10 are intended to be within the scope of the invention. For example, a mouse hVR-2 cDNA can be identified based on the nucleotide sequence of the human VR-2 (hVR-2) or the rat VR-2 (rVR-2).

20 Nucleic acid molecules corresponding to natural allelic variants and homologues of the hVR-1, hVR-2, and rVR-2 cDNAs of the invention can be isolated based on their homology to the hVR-1, hVR-2, and rVR-2 nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid
25 molecules corresponding to natural allelic variants and homologues of the hVR-1, hVR-2, and rVR-2 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the hVR-1, hVR-2, and rVR-2 gene.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under
30 stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12. In other embodiment, the nucleic acid is at least

30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or 950 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% identical to each other typically remain

5 hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-

10 limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50°C, preferably at 55°C, more preferably at 60°C, and even more preferably at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10,

15 or 12 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In addition to naturally-occurring allelic variants of the hVR-1, hVR-2, and rVR-2 sequences that may exist in the population, the skilled artisan will further appreciate that

20 changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, thereby leading to changes in the amino acid sequence of the encoded hVR-1, hVR-2, and rVR-2 proteins, without altering the functional ability of the hVR-1, hVR-2, and rVR-2 proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ

25 ID NO:1, 3, 4, 6, 7, 9, 10, or 12. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of hVR-1, hVR-2, and rVR-2 (*e.g.*, the sequence of SEQ ID NO:2, 5, 8, or 11) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the hVR-1, hVR-2, and rVR-2 proteins of the

30 present invention, are predicted to be particularly unamenable to alteration. Furthermore,

additional amino acid residues that are conserved between the hVR-1, hVR-2, and rVR-2 proteins of the present invention and other members of the Capsaicin/Vanilloid receptor family are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules
5 encoding hVR-1, hVR-2, and rVR-2 proteins that contain changes in amino acid residues that are not essential for activity. Such hVR-1, hVR-2, and rVR-2 proteins differ in amino acid sequence from SEQ ID NO:2, 5, 8, or 11, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about
10 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 95%, 98% or more homologous to SEQ ID NO:2, 5, 8, or 11.

An isolated nucleic acid molecule encoding an hVR-1, hVR-2, and rVR-2 protein homologous to the protein of SEQ ID NO:2, 5, 8, or 11 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of
15 SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid
20 residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*,
25 glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an hVR-1, hVR-2, and rVR-2 protein is preferably replaced with
30 another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an hVR-1, hVR-

2, and rVR-2 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for hVR-1, hVR-2, and rVR-2 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12.

5 In an embodiment, a mutant hVR-1, hVR-2, and rVR-2 protein can be assayed for the ability to (1) interact with a non-hVR-1, non-hVR-2, or non-rVR-2 protein molecule, *e.g.*, a vanilloid compound such as capsaicin; (2) modulate intracellular calcium concentration; (3) activate an hVR-1, hVR-2, and rVR-2-dependent signal transduction pathway; or (4) modulate a pain signaling mechanism.

10 In addition to the nucleic acid molecules encoding hVR-1, hVR-2, and rVR-2 proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or
15 complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire hVR-1, hVR-2, and rVR-2 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding hVR-1, hVR-2, and rVR-2. The term
20 "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of hVR-1, hVR-2, and rVR-2). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding hVR-1, hVR-2, and rVR-2. The term "noncoding region" refers to 5' and 3' sequences which flank the
25 coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

 Given the coding strand sequences encoding hVR-1, hVR-2, and rVR-2 disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be
30 complementary to the entire coding region of hVR-1, hVR-2, and rVR-2 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding

or noncoding region of hVR-1, hVR-2, and rVR-2 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of hVR-1, hVR-2, and rVR-2 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (*v*), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (*v*), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)*w*, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an hVR-1, hVR-2, and rVR-2 protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The

hybridization can be by conventional nucleotide complementarity to form a stable duplex. or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-*o*-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave hVR-1, hVR-2, and rVR-2 mRNA transcripts to thereby inhibit translation of hVR-1, hVR-2, and rVR-2 mRNA. A ribozyme having specificity for an hVR-1, hVR-2, and rVR-2-encoding nucleic acid can be designed based upon the nucleotide sequence of an hVR-1, hVR-2, and rVR-2 cDNA disclosed herein (*i.e.*, SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12). For example, a derivative of a *Tetrahymena* L-19 IVS

RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an hVR-1, hVR-2, and rVR-2-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, hVR-1, hVR-2, and rVR-2 mRNA can be
5 used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, for example, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, hVR-1, hVR-2, and rVR-2 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the hVR-1, hVR-2, and rVR-2 (*e.g.*, the hVR-1, hVR-2, and rVR-2 promoter and/or enhancers) to
10 form triple helical structures that prevent transcription of the hVR-1, hVR-2, and rVR-2 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

15 In yet another embodiment, the hVR-1, hVR-2, and rVR-2 nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996)
20 *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic
25 strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs of hVR-1, hVR-2, and rVR-2 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or
30 antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of hVR-1,

hVR-2, and rVR-2 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. 5 *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of hVR-1, hVR-2, and rVR-2 can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras 10 of hVR-1, hVR-2, and rVR-2 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms 15 of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the 20 PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' 25 PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; 30 PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-

triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

5

II. Isolated hVR-1, hVR-2, and rVR-2 Proteins and Anti-hVR-1, Anti-hVR-2, and Anti-rVR-2 Antibodies

One aspect of the invention pertains to isolated hVR-1, hVR-2, and rVR-2 proteins, and biologically active portions thereof, as well as polypeptide fragments
10 suitable for use as immunogens to raise anti-hVR-2, anti-hVR-2, and anti-rVR-2 antibodies. In one embodiment, native hVR-1, hVR-2, and rVR-2 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, hVR-1, hVR-2, and rVR-2 proteins are produced by recombinant DNA techniques. Alternative to
15 recombinant expression, an hVR-1, hVR-2, and rVR-2 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the hVR-1, hVR-2, and rVR-2 protein is derived, or
20 substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of hVR-1, hVR-2, and rVR-2 protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations
25 of hVR-1, hVR-2, and rVR-2 protein having less than about 30% (by dry weight) of non-hVR-1, hVR-2, and rVR-2 protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-hVR-1, hVR-2, and rVR-2 protein, still more preferably less than about 10% of non-hVR-1, hVR-2, and rVR-2 protein, and most preferably less than about 5% non-hVR-1, non-hVR-2, and non-rVR-2
30 protein. When the hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture

medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of hVR-1, hVR-2, and rVR-2 protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of hVR-1, hVR-2, and rVR-2 protein having less than about 30% (by dry weight) of chemical precursors or non-hVR-1, hVR-2, and rVR-2 chemicals, more preferably less than about 20% chemical precursors or non-hVR-1, hVR-2, and rVR-2 chemicals, still more preferably less than about 10% chemical precursors or non-hVR-1, hVR-2, and rVR-2 chemicals, and most preferably less than about 5% chemical precursors or non-hVR-1, hVR-2, and rVR-2 chemicals.

As used herein, a "biologically active portion" of an hVR-1, hVR-2, and rVR-2 protein includes a fragment of an hVR-1, hVR-2, and rVR-2 protein which participates in an interaction between an hVR-1, hVR-2, and rVR-2 molecule and a non-hVR-1, non-hVR-2, and non-rVR-2 molecule, respectively. Biologically active portions of an hVR-1, hVR-2, and rVR-2 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the hVR-1, hVR-2, and rVR-2 protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, 5, 8, or 11, which include less amino acids than the full length hVR-1, hVR-2, and rVR-2 proteins, and exhibit at least one activity of an hVR-1, hVR-2, and rVR-2 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the hVR-1, hVR-2, and rVR-2 protein, *e.g.*, binding of an hVR-1, hVR-2, and rVR-2 ligand such as a vanilloid compound, *e.g.*, Capsaicin. A biologically active portion of an hVR-1, hVR-2, and rVR-2 protein can be a polypeptide which is, for example, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200 or more amino acids in length. Biologically active portions of an hVR-1, hVR-2, and rVR-2 protein can be used as targets for developing agents which modulate an hVR-1, hVR-2, and rVR-2 mediated activity, *e.g.*, a pain signaling mechanism.

In one embodiment, a biologically active portion of an hVR-1, hVR-2, and rVR-2 protein comprises at least one transmembrane domain, and/or at least one proline rich domain, and/or at least one ankyrin repeat domain. It is to be understood that a biologically active portion of an hVR-1, hVR-2, and rVR-2 protein of the present invention may contain at least one of the above-identified structural domains. A more biologically active portion of an hVR-1, hVR-2, and rVR-2 protein may contain at least two of the above-identified structural domains. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native hVR-1, hVR-2, and rVR-2 protein.

In a embodiment, the hVR-1, hVR-2, and rVR-2 protein has an amino acid sequence shown in SEQ ID NO:2, 5, 8, or 11. In other embodiments, the hVR-1, hVR-2, and rVR-2 protein is substantially homologous to SEQ ID NO:2, 5, 8, or 11, and retains the functional activity of the protein of SEQ ID NO:2, 5, 8, or 11, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the hVR-1, hVR-2, and rVR-2 protein is a protein which comprises an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 95%, 98% or more homologous to SEQ ID NO:2, 5, 8, or 11.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the hVR-1, hVR-2, and rVR-2 amino acid sequence of SEQ ID NO:2, 5, 8, or 11, having 177 amino acid residues, at least 80, preferably at least 100, more preferably at least 120, even more preferably at least 140, and even more preferably at least 150, 160 or 170 amino acid residues are aligned). The amino acid residues or nucleotides at

corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to hVR-1, hVR-2, and rVR-2 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50,

wordlength = 3 to obtain amino acid sequences homologous to hVR-1, hVR-2, and rVR-2 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, 5 the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

The invention also provides hVR-1, hVR-2, and rVR-2 chimeric or fusion proteins. As used herein, an hVR-1, hVR-2, and rVR-2 "chimeric protein" or "fusion protein" comprises an hVR-1, hVR-2, and rVR-2 polypeptide operatively linked to a 10 non-hVR-1, hVR-2, and rVR-2 polypeptide. An "hVR-1, hVR-2, and rVR-2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to hVR-1, hVR-2, and rVR-2, whereas a "non-hVR-1, non-hVR-2, and non-rVR-2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the hVR-1, hVR-2, and rVR-2 protein, 15 *e.g.*, a protein which is different from the hVR-1, hVR-2, and rVR-2 protein and which is derived from the same or a different organism. Within an hVR-1, hVR-2, and rVR-2 fusion protein the hVR-1, hVR-2, and rVR-2 polypeptide can correspond to all or a portion of an hVR-1, hVR-2, and rVR-2 protein. In a embodiment, an hVR-1, hVR-2, and rVR-2 fusion protein comprises at least one biologically active portion of an hVR-1, 20 hVR-2, and rVR-2 protein. In another embodiment, an hVR-1, hVR-2, and rVR-2 fusion protein comprises at least two biologically active portions of an hVR-1, hVR-2, and rVR-2 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the hVR-1, hVR-2, and rVR-2 polypeptide and the non-hVR-1, non-hVR-2, and non-rVR-2 polypeptide are fused in-frame to each other. The non-hVR-1, 25 hVR-2, and rVR-2 polypeptide can be fused to the N-terminus or C-terminus of the hVR-1, hVR-2, and rVR-2 polypeptide.

For example, in one embodiment, the fusion protein is a GST-hVR-1, GST-hVR-2, and GST-rVR-2 fusion protein in which the hVR-1, hVR-2, and rVR-2 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can 30 facilitate the purification of recombinant hVR-1, hVR-2, and rVR-2.

In another embodiment, the fusion protein is an hVR-1, hVR-2, and rVR-2 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of hVR-1, hVR-2, and rVR-2 can be increased through use of a heterologous signal sequence.

5 The hVR-1, hVR-2, and rVR-2 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The hVR-1, hVR-2, and rVR-2 fusion proteins can be used to affect the bioavailability of an hVR-1, hVR-2, and rVR-2 substrate. Use of hVR-1, hVR-2, and rVR-2 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for
10 example, (i) aberrant modification or mutation of a gene encoding an hVR-1, hVR-2, and rVR-2 protein; (ii) mis-regulation of the hVR-1, hVR-2, and rVR-2 gene; and (iii) aberrant post-translational modification of an hVR-1, hVR-2, and rVR-2 protein.

Moreover, the hVR-1, hVR-2, and rVR-2-fusion proteins of the invention can be used as immunogens to produce anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies in a
15 subject, to purify hVR-1, hVR-2, and rVR-2 ligands and in screening assays to identify molecules which inhibit the interaction of hVR-1, hVR-2, and rVR-2 with an hVR-1, hVR-2, and rVR-2 substrate.

Preferably, an hVR-1, hVR-2, and rVR-2 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA
20 fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment,
25 the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular*
30 *Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST

polypeptide). An hVR-1, hVR-2, and rVR-2-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the hVR-1, hVR-2, and rVR-2 protein.

The present invention also pertains to variants of the hVR-1, hVR-2, and rVR-2
5 proteins which function as either hVR-1, hVR-2, and rVR-2 agonists (mimetics) or as hVR-1, hVR-2, and rVR-2 antagonists. Variants of the hVR-1, hVR-2, and rVR-2 proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of an hVR-1, hVR-2, and rVR-2 protein. An agonist of the hVR-1, hVR-2, and rVR-2 proteins can retain substantially the same, or a subset, of the biological activities of the
10 naturally occurring form of an hVR-1, hVR-2, and rVR-2 protein. An antagonist of an hVR-1, hVR-2, and rVR-2 protein can inhibit one or more of the activities of the naturally occurring form of the hVR-1, hVR-2, and rVR-2 protein by, for example, competitively modulating an hVR-1, hVR-2, and rVR-2-mediated activity of an hVR-1, hVR-2, and rVR-2 protein. Thus, specific biological effects can be elicited by treatment
15 with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the hVR-1, hVR-2, and rVR-2 protein.

In one embodiment, variants of an hVR-1, hVR-2, and rVR-2 protein which
20 function as either hVR-1, hVR-2, and rVR-2 agonists (mimetics) or as hVR-1, hVR-2, and rVR-2 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of an hVR-1, hVR-2, and rVR-2 protein for hVR-1, hVR-2, and rVR-2 protein agonist or antagonist activity. In one embodiment, a variegated library of hVR-1, hVR-2, and rVR-2 variants is generated by combinatorial mutagenesis at the
25 nucleic acid level and is encoded by a variegated gene library. A variegated library of hVR-1, hVR-2, and rVR-2 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential hVR-1, hVR-2, and rVR-2 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage
30 display) containing the set of hVR-1, hVR-2, and rVR-2 sequences therein. There are a variety of methods which can be used to produce libraries of potential hVR-1, hVR-2,

and rVR-2 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential hVR-1, hVR-2, and rVR-2 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of an hVR-1, hVR-2, and rVR-2 protein coding sequence can be used to generate a variegated population of hVR-1, hVR-2, and rVR-2 fragments for screening and subsequent selection of variants of an hVR-1, hVR-2, and rVR-2 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an hVR-1, hVR-2, and rVR-2 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the hVR-1, hVR-2, and rVR-2 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of hVR-1, hVR-2, and rVR-2 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis

(REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify hVR-1, hVR-2, and rVR-2 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

5 In one embodiment, cell based assays can be exploited to analyze a variegated hVR-1, hVR-2, and rVR-2 library. For example, a library of expression vectors can be transfected into a cell line, *e.g.*, a neuronal cell line, which ordinarily responds to a particular ligand in an hVR-1, hVR-2, and rVR-2-dependent manner. The transfected cells are then contacted with the ligand and the effect of expression of the mutant on
10 signaling by the ligand can be detected, *e.g.*, by measuring intracellular calcium concentration, neuronal membrane depolarization, or the activity of an hVR-1, hVR-2, and rVR-2-regulated transcription factor. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the ligand, and the individual clones further characterized.

15 An isolated hVR-1, hVR-2, and rVR-2 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind hVR-1, hVR-2, and rVR-2 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length hVR-1, hVR-2, and rVR-2 protein can be used or, alternatively, the invention provides antigenic peptide fragments of hVR-1, hVR-2, and rVR-2 for use as
20 immunogens. The antigenic peptide of hVR-1, hVR-2, and rVR-2 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, 5, 8, or 11 and encompasses an epitope of hVR-1, hVR-2, and rVR-2 such that an antibody raised against the peptide forms a specific immune complex with hVR-1, hVR-2, and rVR-2. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more
25 preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

 Epitopes encompassed by the antigenic peptide are regions of hVR-1, hVR-2, and rVR-2 that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity (see, for example, Figures 12 and 14).

An hVR-1, hVR-2, and rVR-2 immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed hVR-1, hVR-2, and rVR-2 protein or a chemically synthesized hVR-1, hVR-2, and rVR-2 polypeptide. The preparation can further include an
5 adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic hVR-1, hVR-2, and rVR-2 preparation induces a polyclonal anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody response.

Accordingly, another aspect of the invention pertains to anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as hVR-1, hVR-2, and rVR-2. Examples of
15 immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind hVR-1, hVR-2, and rVR-2. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain
20 only one species of an antigen binding site capable of immunoreacting with a particular epitope of hVR-1, hVR-2, and rVR-2. A monoclonal antibody composition thus typically displays a single binding affinity for a particular hVR-1, hVR-2, and rVR-2 protein with which it immunoreacts.

Polyclonal anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies can be prepared
25 as described above by immunizing a suitable subject with an hVR-1, hVR-2, and rVR-2 immunogen. The anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized hVR-1, hVR-2, and rVR-2. If desired, the antibody molecules directed against hVR-1, hVR-2, and rVR-2
30 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an

appropriate time after immunization, *e.g.*, when the anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:23136). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an hVR-1, hVR-2, and rVR-2 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds hVR-1, hVR-2, and rVR-2.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-hVR-1, anti-hVR-2, and anti-rVR-2 monoclonal antibodies (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard

techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which
5 kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind hVR-1, hVR-2, and rVR-2, *e.g.*, using a standard ELISA assay.

10 Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with hVR-1, hVR-2, and rVR-2 to thereby isolate immunoglobulin library members that bind hVR-1, hVR-2, and rVR-2. Kits for
15 generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No.
20 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International
25 Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA*
30 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.*

(1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.

Additionally, recombinant anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody (*e.g.*, monoclonal antibody) can be used to isolate hVR-1, hVR-2, and rVR-2 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody can facilitate the purification of natural hVR-1, hVR-2, and rVR-2 from cells and of recombinantly produced hVR-1, hVR-2, and rVR-2 expressed in host cells. Moreover, an anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody can be used to detect hVR-1, hVR-2, and rVR-2 protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the hVR-1, hVR-2, and rVR-2 protein. Anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can

be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, 5 alkaline phosphatase, -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of 10 bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression 15 vectors, containing a nucleic acid encoding an hVR-1, hVR-2, and rVR-2 protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, 20 wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated 25 along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most 30 commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective

retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, hVR-1, hVR-2, and rVR-2 proteins, mutant forms of hVR-1, hVR-2, and rVR-2 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of hVR-1, hVR-2, and rVR-2 proteins in prokaryotic or eukaryotic cells. For example, hVR-1, hVR-2, and rVR-2 proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the

recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in hVR-1, hVR-2, and rVR-2 activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to, for example, generate antibodies specific for hVR-1, hVR-2, and rVR-2 proteins. In a embodiment, an hVR-1, hVR-2, and rVR-2 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid *trp-lac* fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 *gn10-lac* fusion promoter mediated by a coexpressed viral RNA polymerase (T7 *gn1*). This viral

polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 *gn1* gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express
5 the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those
10 preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the hVR-1, hVR-2, and rVR-2 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include
15 pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

Alternatively, hVR-1, hVR-2, and rVR-2 proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression
20 of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian
25 expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both
30 prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring

Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The expression characteristics of an endogenous hVR-1, hVR-2, and rVR-2 gene within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous hVR-1, hVR-2, and rVR-2 gene. For example, an endogenous hVR-1, hVR-2, and rVR-2 gene which is normally "transcriptionally silent", i.e., a hVR-1, hVR-2, and rVR-2 gene which is normally not expressed, or is expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a transcriptionally silent, endogenous hVR-1, hVR-2, and rVR-2 gene, may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous hVR-1, hVR-2, and rVR-2 gene, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described *e.g.*, in Chappel, U.S.

5 Patent No.: 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an
10 RNA molecule which is antisense to hVR-1, hVR-2, and rVR-2 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific
15 expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*,
20 Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which an hVR-1, hVR-2, and rVR-2 nucleic acid molecule of the invention is introduced, *e.g.*, an hVR-1, hVR-2, and rVR-2 nucleic acid molecule within a recombinant expression vector or an hVR-
25 1, hVR-2, and rVR-2 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding
30 generations due to either mutation or environmental influences, such progeny may not,

in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, an hVR-1, hVR-2, and rVR-2 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding an hVR-1, hVR-2, and rVR-2 protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) an hVR-1, hVR-2, and rVR-2 protein. Accordingly, the invention further provides methods for producing an hVR-1, hVR-2, and rVR-2 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression

vector encoding an hVR-1, hVR-2, and rVR-2 protein has been introduced) in a suitable medium such that an hVR-1, hVR-2, and rVR-2 protein is produced. In another embodiment, the method further comprises isolating an hVR-1, hVR-2, and rVR-2 protein from the medium or the host cell.

5 The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which hVR-1, hVR-2, and rVR-2-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous hVR-1, hVR-2, and rVR-2 sequences have been
10 introduced into their genome or homologous recombinant animals in which endogenous hVR-1, hVR-2, and rVR-2 sequences have been altered. Such animals are useful for studying the function and/or activity of an hVR-1, hVR-2, and rVR-2 and for identifying and/or evaluating modulators of hVR-1, hVR-2, and rVR-2 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a
15 rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression
20 of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous hVR-1, hVR-2, and rVR-2 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an
25 embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing an hVR-1, hVR-2, and rVR-2-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The hVR-1, hVR-2, and rVR-2 cDNA sequence
30 of SEQ ID NO:1, 3, 5, 7 or 9 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a hVR-2 gene, such as a

mouse or rat hVR-2, *e.g.*, the rVR-2 gene, can be used as a transgene. Alternatively, an hVR-1, hVR-2, and rVR-2 gene homologue, such as another member of the Capsaicin/Vanilloid family, can be isolated based on hybridization to the hVR-1, hVR-2, and rVR-2 cDNA sequences of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, (described further
5 in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to an hVR-1, hVR-2, and rVR-2 transgene to direct expression of an hVR-1, hVR-2, and rVR-2 protein to particular cells. Methods for generating transgenic animals via embryo
10 manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other
15 transgenic animals. A transgenic founder animal can be identified based upon the presence of an hVR-1, hVR-2, and rVR-2 transgene in its genome and/or expression of hVR-1, hVR-2, and rVR-2 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding an hVR-1, hVR-2, and
20 rVR-2 protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an hVR-1, hVR-2, and rVR-2 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the hVR-1, hVR-2, and rVR-2 gene. The VR-1 or VR-2 gene can be a human gene (*e.g.*, the cDNA
25 of SEQ ID NO:1, 3, 5, 4, 6, 7, or 9), but more preferably, is a non-human homologue of a hVR-1 and hVR-2 gene (*e.g.*, the cDNA of SEQ ID NO:10 or 12, or a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO: 1, 3, 5, 4, 6, 7, or 9). For example, a mouse VR-2 gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous
30 VR-2 gene in the mouse genome. In a embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the

endogenous hVR-1, hVR-2, and rVR-2 gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous hVR-1, hVR-2, and rVR-2 gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous hVR-1, hVR-2, and rVR-2 protein). In the homologous recombination nucleic acid molecule, the altered portion of the hVR-1, hVR-2, and rVR-2 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the hVR-1, hVR-2, and rVR-2 gene to allow for homologous recombination to occur between the exogenous hVR-1, hVR-2, and rVR-2 gene carried by the homologous recombination nucleic acid molecule and an endogenous hVR-1, hVR-2, and rVR-2 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking hVR-1, hVR-2, and rVR-2 nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced hVR-1, hVR-2, and rVR-2 gene has homologously recombined with the endogenous hVR-1, hVR-2, and rVR-2 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT

International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One
5 example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the
10 transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

15 Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through
20 the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

25

IV. Pharmaceutical Compositions

The hVR-1, hVR-2, and rVR-2 nucleic acid molecules, fragments of hVR-1, hVR-2, and rVR-2 proteins, and anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into
30 pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically

acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for

example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of
5 surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be
10 brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a fragment of an hVR-1, hVR-2, and rVR-2 protein or an anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibody) in the required amount in an appropriate solvent
15 with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum
20 drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and
25 used in the form of tablets, troches, or capsules. oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the
30 following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or

lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

- 5 For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be
10 permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in
15 the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

- In one embodiment, the active compounds are prepared with carriers that will
20 protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.
- 25 The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound
5 calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

10 Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio
15 LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in
20 formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially
25 from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high
30 performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon

the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

5 Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the
10 potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein.

When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example,
15 prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration,
20 the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by
25 stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the
30 pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

5 The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, an hVR-1, hVR-2, and rVR-2 protein of the
10 invention has one or more of the following activities: (1) it interacts with a non-hVR-1, non-hVR-2, and non-rVR-2 protein molecule, *e.g.*, a vanilloid compound such as capsaicin; (2) it modulates intracellular calcium concentration; (3) it activates an hVR-1, hVR-2, and rVR-2-dependent signal transduction pathway; and (4) it modulates a pain signaling mechanism, and, thus, can be used to, for example, (1) modulate the
15 interaction with a non-hVR-1, non-hVR-2, and non-rVR-2 protein molecule; (2) modulate intracellular calcium concentration; (3) activate an hVR-1, hVR-2, and rVR-2-dependent signal transduction pathway; and (4) modulate a pain signaling mechanism.

 The isolated nucleic acid molecules of the invention can be used, for example, to express hVR-1, hVR-2, and rVR-2 protein (*e.g.*, via a recombinant expression vector in
20 a host cell in gene therapy applications), to detect hVR-1, hVR-2, and rVR-2 mRNA (*e.g.*, in a biological sample) or a genetic alteration in an hVR-1, hVR-2, and rVR-2 gene, and to modulate hVR-1, hVR-2, and rVR-2 activity, as described further below. The hVR-1, hVR-2, and rVR-2 proteins can be used to screen for naturally occurring hVR-1, hVR-2, and rVR-2 substrates, to screen for drugs or compounds which modulate
25 hVR-1, hVR-2, and rVR-2 activity, as well as to treat disorders characterized by insufficient or excessive production of hVR-1, hVR-2, and rVR-2 protein or production of hVR-1, hVR-2, and rVR-2 protein forms which have decreased or aberrant activity compared to hVR-1, hVR-2, and rVR-2 wild type protein (*e.g.*, pain disorders). Moreover, the anti-hVR-1, anti-hVR-2, and anti-rVR-2 antibodies of the invention can
30 be used to detect and isolate hVR-1, hVR-2, and rVR-2 proteins, regulate the

bioavailability of hVR-1, hVR-2, and rVR-2 proteins, and modulate hVR-1, hVR-2, and rVR-2 activity.

A. Screening Assays:

5 The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to hVR-1, hVR-2, and rVR-2 proteins, have a stimulatory or inhibitory effect on, for example, hVR-1, hVR-2, and rVR-2 expression or hVR-1, hVR-2, and rVR-2 activity, or have a stimulatory or
10 inhibitory effect on, for example, the expression or activity of hVR-1, hVR-2, and rVR-2 substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of an hVR-1, hVR-2, and rVR-2 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides
15 assays for screening candidate or test compounds which bind to or modulate the activity of an hVR-1, hVR-2, and rVR-2 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries;
20 synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

25 Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in
30 Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage
5 (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell, *e.g.*, a neuronal cell, which expresses an hVR-1, hVR-2, and rVR-2 protein or biologically
10 active portion thereof is contacted with a test compound and the ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 activity is determined. Determining the ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 activity can be accomplished by monitoring, for example, intracellular calcium concentration or membrane depolarization by, *e.g.*, patch-clamp recordings in whole-cell, inside-out, and
15 outside-out configurations (as described in, for example, Tominaga M. *et al.* (1998) *Neuron* 21:531-543). Determining the ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 activity can further be accomplished by monitoring the activity of an hVR-1, hVR-2, and rVR-2-regulated transcription factor. The cell, for example, can be of mammalian origin, *e.g.*, a neuronal cell.

20 The ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 binding to a substrate or to bind to hVR-1, hVR-2, and rVR-2 can also be determined. Determining the ability of the test compound to modulate hVR-1, hVR-2, and rVR-2 binding to a substrate can be accomplished, for example, by coupling the hVR-1, hVR-2, and rVR-2 substrate with a radioisotope or enzymatic label such that binding of the
25 hVR-1, hVR-2, and rVR-2 substrate to hVR-1, hVR-2, and rVR-2 can be determined by detecting the labeled hVR-1, hVR-2, and rVR-2 substrate in a complex. Determining the ability of the test compound to bind hVR-1, hVR-2, and rVR-2 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to hVR-1, hVR-2, and rVR-2 can be determined by detecting
30 the labeled hVR-1, hVR-2, and rVR-2 compound in a complex. For example, compounds (*e.g.*, hVR-1, hVR-2, and rVR-2 substrates) can be labeled with ¹²⁵I, ³⁵S,

^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, an hVR-1, hVR-2, and rVR-2 substrate) to interact with hVR-1, hVR-2, and rVR-2 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with hVR-1, hVR-2, and rVR-2 without the labeling of either the compound or the hVR-1, hVR-2, and rVR-2. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and hVR-1, hVR-2, and rVR-2.

In yet another embodiment, an assay of the present invention is a cell-free assay in which an hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is determined. biologically active portions of the hVR-1, hVR-2, and rVR-2 proteins to be used in assays of the present invention include fragments which participate in interactions with non-hVR-1, non-hVR-2, and non-rVR-2 molecules, *e.g.*, fragments with high surface probability scores. Binding of the test compound to the hVR-1, hVR-2, and rVR-2 protein can be determined either directly or indirectly as described above. In a embodiment, the assay includes contacting the hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof with a known compound which binds hVR-1, hVR-2, and rVR-2 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an hVR-1, hVR-2, and rVR-2 protein, wherein determining the ability of the test compound to interact with an hVR-1, hVR-2, and rVR-2 protein comprises determining the ability of the test

compound to preferentially bind to hVR-1, hVR-2, and rVR-2 or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which an hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of an hVR-1, hVR-2, and rVR-2 protein can be accomplished, for example, by determining the ability of the hVR-1, hVR-2, and rVR-2 protein to bind to an hVR-1, hVR-2, and rVR-2 target molecule, *e.g.*, a vanilloid compound such as capsaicin, by one of the methods described above for determining direct binding. Determining the ability of the hVR-1, hVR-2, and rVR-2 protein to bind to an hVR-1, hVR-2, and rVR-2 target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of an hVR-1, hVR-2, and rVR-2 protein can be accomplished by determining the ability of the hVR-1, hVR-2, and rVR-2 protein to further modulate the activity of a downstream effector of an hVR-1, hVR-2, and rVR-2 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting an hVR-1, hVR-2, and rVR-2 protein or biologically active portion thereof with a known compound which binds the hVR-1, hVR-2, and rVR-2 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the hVR-1, hVR-2, and rVR-2 protein, wherein

determining the ability of the test compound to interact with the hVR-1, hVR-2, and rVR-2 protein comprises determining the ability of the hVR-1, hVR-2, and rVR-2 protein to preferentially bind to or modulate the activity of an hVR-1, hVR-2, and rVR-2 target molecule.

5 The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of isolated proteins (*e.g.*, hVR-1, hVR-2, and rVR-2 proteins or biologically active portions thereof). In the case of cell-free assays in which a membrane-bound form of an isolated protein is used it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is
10 maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, 3-[(3-
15 cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-
cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

 In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either hVR-1, hVR-2, and rVR-2 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or
20 both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to an hVR-1, hVR-2, and rVR-2 protein, or interaction of an hVR-1, hVR-2, and rVR-2 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-
25 centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ hVR-1, hVR-2, and rVR-2 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are
30 then combined with the test compound or the test compound and either the non-adsorbed target protein or hVR-1, hVR-2, and rVR-2 protein, and the mixture incubated under

conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of hVR-1, hVR-2, and rVR-2 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either an hVR-1, hVR-2, and rVR-2 protein or an hVR-1, hVR-2, and rVR-2 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated hVR-1, hVR-2, and rVR-2 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with hVR-1, hVR-2, and rVR-2 protein or target molecules but which do not interfere with binding of the hVR-1, hVR-2, and rVR-2 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or hVR-1, hVR-2, and rVR-2 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the hVR-1, hVR-2, and rVR-2 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the hVR-1, hVR-2, and rVR-2 protein or target molecule.

In another embodiment, modulators of hVR-1, hVR-2, and rVR-2 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of hVR-1, hVR-2, and rVR-2 mRNA or protein in the cell is determined. The level of expression of hVR-1, hVR-2, and rVR-2 mRNA or protein in the presence of the candidate compound is compared to the level of expression of hVR-1, hVR-2, and rVR-2 mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of hVR-1, hVR-2, and rVR-2 expression based on this comparison. For example, when expression of hVR-1, hVR-2, and rVR-2 mRNA or protein is greater (statistically significantly greater) in the presence

of the candidate compound than in its absence, the candidate compound is identified as a stimulator of hVR-1, hVR-2, and rVR-2 mRNA or protein expression. Alternatively, when expression of hVR-1, hVR-2, and rVR-2 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of hVR-1, hVR-2, and rVR-2 mRNA or protein expression. The level of hVR-1, hVR-2, and rVR-2 mRNA or protein expression in the cells can be determined by methods described herein for detecting hVR-1, hVR-2, and rVR-2 mRNA or protein.

In yet another aspect of the invention, the hVR-1, hVR-2, and rVR-2 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with hVR-1, hVR-2, and rVR-2 ("hVR-1-binding proteins", "hVR-2-binding proteins", and "rVR-2-binding proteins" or "hVR-1-bp", "hVR-2-bp", and "rVR-2-bp") and are involved in hVR-1, hVR-2, and rVR-2 activity. Such hVR-1, hVR-2, and rVR-2-binding proteins are also likely to be involved in the propagation of signals by the hVR-1, hVR-2, and rVR-2 proteins or hVR-1, hVR-2, and rVR-2 targets as, for example, downstream elements of an hVR-1, hVR-2, and rVR-2-mediated signaling pathway, *e.g.*, a pain signaling pathway. Alternatively, such hVR-1, hVR-2, and rVR-2-binding proteins are likely to be hVR-1, hVR-2, and rVR-2 inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for an hVR-1, hVR-2, and rVR-2 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an hVR-1, hVR-2, and rVR-2-dependent complex, the DNA-binding and

activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the hVR-1, hVR-2, and rVR-2 protein.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, an hVR-1, hVR-2, and rVR-2 modulating agent, an antisense hVR-1, hVR-2, and rVR-2 nucleic acid molecule, an hVR-1, hVR-2, and rVR-2-specific antibody, or an hVR-1, hVR-2, and rVR-2-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the hVR-1, hVR-2,

and rVR-2 nucleotide sequences, described herein, can be used to map the location of the hVR-1, hVR-2, and rVR-2 genes on a chromosome. The mapping of the hVR-1, hVR-2, and rVR-2 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

5 Briefly, hVR-1, hVR-2, and rVR-2 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the hVR-1, hVR-2, and rVR-2 nucleotide sequences. Computer analysis of the hVR-1, hVR-2, and rVR-2 sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be
10 used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the hVR-1, hVR-2, and rVR-2 sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow
15 and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single
20 human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

25 PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the hVR-1, hVR-2, and rVR-2 nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which
30 can similarly be used to map an hVR-1, hVR-2, and rVR-2 sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci.*

USA, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the hVR-1, hVR-2, and rVR-2 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not

in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The hVR-1, hVR-2, and rVR-2 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the hVR-1, hVR-2, and rVR-2 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The hVR-1, hVR-2, and rVR-2 nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding

regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals.

If a panel of reagents from hVR-1, hVR-2, and rVR-2 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of Partial hVR-1, hVR-2, and rVR-2 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Examples of polynucleotide reagents include the hVR-1, hVR-2, and rVR-2 nucleotide sequences or portions thereof, *e.g.*, fragments derived from SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 11 having a length of at least 20 bases, preferably at least 30 bases.

The hVR-1, hVR-2, and rVR-2 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is
5 presented with a tissue of unknown origin. Panels of such hVR-1, hVR-2, and rVR-2 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, hVR-1, hVR-2, and rVR-2 primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

10

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically.
15 Accordingly, one aspect of the present invention relates to diagnostic assays for determining hVR-1, hVR-2, and rVR-2 protein and/or nucleic acid expression as well as hVR-1, hVR-2, and rVR-2 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant hVR-
20 1, hVR-2, and rVR-2 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with hVR-1, hVR-2, and rVR-2 protein, nucleic acid expression or activity. For example, mutations in an hVR-1, hVR-2, and rVR-2 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to
25 thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with hVR-1, hVR-2, and rVR-2 protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of hVR-1, hVR-2, and rVR-2 in
30 clinical trials.

These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of hVR-1, hVR-2, and rVR-2 protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting hVR-1, hVR-2, and rVR-2 protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes hVR-1, hVR-2, and rVR-2 protein such that the presence of hVR-1, hVR-2, and rVR-2 protein or nucleic acid is detected in the biological sample. A agent for detecting hVR-1, hVR-2, and rVR-2 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to hVR-1, hVR-2, and rVR-2 mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length hVR-1, hVR-2, and rVR-2 nucleic acid, such as the nucleic acid of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to hVR-1, hVR-2, and rVR-2 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting hVR-1, hVR-2, and rVR-2 protein is an antibody capable of binding to hVR-1, hVR-2, and rVR-2 protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect hVR-1, hVR-2, and rVR-2 mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of hVR-1, hVR-2, and rVR-2 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro*

techniques for detection of hVR-1, hVR-2, and rVR-2 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of hVR-1, hVR-2, and rVR-2 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for
5 detection of hVR-1, hVR-2, and rVR-2 protein include introducing into a subject a labeled anti-hVR-1, hVR-2, and rVR-2 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the
10 test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a
15 compound or agent capable of detecting hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA, such that the presence of hVR-1, hVR-2, and rVR-2 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of hVR-1, hVR-2, and rVR-2 protein, mRNA or genomic DNA in the control sample with the presence of hVR-1, hVR-2, and rVR-2 protein, mRNA or genomic DNA in the test
20 sample.

The invention also encompasses kits for detecting the presence of hVR-1, hVR-2, and rVR-2 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting hVR-1, hVR-2, and rVR-2 protein or mRNA in a biological sample; means for determining the amount of hVR-1, hVR-2, and rVR-2 in
25 the sample; and means for comparing the amount of hVR-1, hVR-2, and rVR-2 in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect hVR-1, hVR-2, and rVR-2 protein or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity. As used herein, the term "aberrant" includes an hVR-1, hVR-2, and rVR-2 expression or activity which deviates from the wild type hVR-1, hVR-2, and rVR-2 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant hVR-1, hVR-2, and rVR-2 expression or activity is intended to include the cases in which a mutation in the hVR-1, hVR-2, and rVR-2 gene causes the hVR-1, hVR-2, and rVR-2 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional hVR-1, hVR-2, and rVR-2 protein or a protein which does not function in a wild-type fashion, *e.g.*, a protein which does not interact with an hVR-1, hVR-2, and rVR-2 ligand or one which interacts with a non-hVR-1, non-hVR-2, and non-rVR-2 ligand.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in hVR-1, hVR-2, and rVR-2 protein activity or nucleic acid expression, such as a pain disorder. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in hVR-1, hVR-2, and rVR-2 protein activity or nucleic acid expression, such as a pain disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity in which a test sample is obtained from a subject and hVR-1, hVR-2, and rVR-2 protein or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of hVR-1, hVR-2, and rVR-2 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a pain disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity in which a test sample is obtained and hVR-1, hVR-2, and rVR-2 protein or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of hVR-1, hVR-2, and rVR-2 protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity).

The methods of the invention can also be used to detect genetic alterations in an hVR-1, hVR-2, and rVR-2 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in hVR-1, hVR-2, and rVR-2 protein activity or nucleic acid expression, such as a neurodegenerative disorder. In embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding an hVR-1, hVR-2, and rVR-2-protein, or the mis-expression of the hVR-1, hVR-2, and rVR-2 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from an hVR-1, hVR-2, and rVR-2 gene; 2) an addition of one or more nucleotides to an hVR-1, hVR-2, and rVR-2 gene; 3) a substitution of one or more nucleotides of an hVR-1, hVR-2, and rVR-2 gene, 4) a chromosomal rearrangement of an hVR-1, hVR-2, and rVR-2 gene; 5) an alteration in the level of a messenger RNA transcript of an hVR-1, hVR-2, and rVR-2 gene, 6) aberrant modification of an hVR-1, hVR-2, and rVR-2 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of an hVR-1, hVR-2, and rVR-2 gene, 8) a non-wild type level of an hVR-1, hVR-2, and rVR-2-protein, 9) allelic loss of an hVR-1, hVR-2, and rVR-2 gene,

and 10) inappropriate post-translational modification of an hVR-1, hVR-2, and rVR-2-protein. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in an hVR-1, hVR-2, and rVR-2 gene. A biological sample is a tissue or serum sample isolated by conventional means from a subject.

5 In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which
10 can be particularly useful for detecting point mutations in the hVR-1, hVR-2, and rVR-2-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to an hVR-1, hVR-2, and
15 rVR-2 gene under conditions such that hybridization and amplification of the hVR-1, hVR-2, and rVR-2-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used
20 for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any
25 other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an hVR-1, hVR-2, and rVR-2 gene
30 from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally),

digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in hVR-1, hVR-2, and rVR-2 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in hVR-1, hVR-2, and rVR-2 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the hVR-1, hVR-2, and rVR-2 gene and detect mutations by comparing the sequence of the sample hVR-1, hVR-2, and rVR-2 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the hVR-1, hVR-2, and rVR-2 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing
5 heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type hVR-1, hVR-2, and rVR-2 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA
10 duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing
15 polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called
20 "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in hVR-1, hVR-2, and rVR-2 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based
25 on an hVR-1, hVR-2, and rVR-2 sequence, *e.g.*, a wild-type hVR-1, hVR-2, and rVR-2 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in hVR-1, hVR-2, and rVR-2 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control hVR-1, hVR-2, and rVR-2 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different

mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention.

- 5 Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable
10 to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect
15 the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose
20 patients exhibiting symptoms or family history of a disease or illness involving an hVR-1, hVR-2, and rVR-2 gene.

Furthermore, any cell type or tissue in which hVR-1, hVR-2, and rVR-2 is expressed may be utilized in the prognostic assays described herein.

25 3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of an hVR-1, hVR-2, and rVR-2 protein can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase hVR-1, hVR-2, and rVR-2 gene
30 expression, protein levels, or upregulate hVR-1, hVR-2, and rVR-2 activity, can be monitored in clinical trials of subjects exhibiting decreased hVR-1, hVR-2, and rVR-2

gene expression, protein levels, or downregulated hVR-1, hVR-2, and rVR-2 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease hVR-1, hVR-2, and rVR-2 gene expression, protein levels, or downregulate hVR-1, hVR-2, and rVR-2 activity, can be monitored in clinical trials of subjects exhibiting
5 increased hVR-1, hVR-2, and rVR-2 gene expression, protein levels, or upregulated hVR-1, hVR-2, and rVR-2 activity. In such clinical trials, the expression or activity of an hVR-1, hVR-2, and rVR-2 gene, and preferably, other genes that have been implicated in, for example, an hVR-1, hVR-2, and rVR-2-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

10 For example, and not by way of limitation, genes, including hVR-1, hVR-2, and rVR-2, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates hVR-1, hVR-2, and rVR-2 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on hVR-1, hVR-2, and rVR-2-associated disorders (*e.g.*, pain disorders), for
15 example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of hVR-1, hVR-2, and rVR-2 and other genes implicated in the hVR-1, hVR-2, and rVR-2-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein
20 produced, by one of the methods as described herein, or by measuring the levels of activity of hVR-1, hVR-2, and rVR-2 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

25 In a embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii)
30 detecting the level of expression of an hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-

administration samples from the subject; (iv) detecting the level of expression or activity of the hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA in the pre-administration sample with the hVR-1, hVR-2, and rVR-2 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of hVR-1, hVR-2, and rVR-2 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of hVR-1, hVR-2, and rVR-2 to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, hVR-1, hVR-2, and rVR-2 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

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D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant hVR-1, hVR-2, and rVR-2 expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the hVR-1, hVR-2, and rVR-2 molecules of the present invention or hVR-1, hVR-2, and rVR-2 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or

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therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. Prophylactic Methods

5 In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant hVR-1, hVR-2, and rVR-2 expression or activity, by administering to the subject an hVR-1, hVR-2, and rVR-2 or an agent which modulates hVR-1, hVR-2, and rVR-2 expression or at least one hVR-1, hVR-2, and rVR-2 activity. Subjects at risk for a disease which is caused or contributed to by
10 aberrant hVR-1, hVR-2, and rVR-2 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the hVR-1, hVR-2, and rVR-2 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of hVR-
15 1, hVR-2, and rVR-2 aberrancy, for example, an hVR-1, hVR-2, and rVR-2, hVR-1, hVR-2, and rVR-2 agonist or hVR-1, hVR-2, and rVR-2 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

20 2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating hVR-1, hVR-2, and rVR-2 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with an hVR-1, hVR-2, and rVR-2 or agent that modulates one or more of the
25 activities of hVR-1, hVR-2, and rVR-2 protein activity associated with the cell. An agent that modulates hVR-1, hVR-2, and rVR-2 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of an hVR-1, hVR-2, and rVR-2 protein (*e.g.*, an hVR-1, hVR-2, and rVR-2 substrate), an hVR-1, hVR-2, and rVR-2 antibody, an hVR-1, hVR-2, and rVR-2 agonist
30 or antagonist, a peptidomimetic of an hVR-1, hVR-2, and rVR-2 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more hVR-1,

hVR-2, and rVR-2 activities. Examples of such stimulatory agents include active hVR-1, hVR-2, and rVR-2 protein and a nucleic acid molecule encoding hVR-1, hVR-2, and rVR-2 that has been introduced into the cell. In another embodiment, the agent inhibits one or more hVR-1, hVR-2, and rVR-2 activities. Examples of such inhibitory agents
5 include antisense hVR-1, hVR-2, and rVR-2 nucleic acid molecules, anti-hVR-1, hVR-2, and rVR-2 antibodies, and hVR-1, hVR-2, and rVR-2 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder
10 characterized by aberrant expression or activity of an hVR-1, hVR-2, and rVR-2 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) hVR-1, hVR-2, and rVR-2 expression or activity. In another embodiment, the method involves administering an
15 hVR-1, hVR-2, and rVR-2 protein or nucleic acid molecule as therapy to compensate for reduced or aberrant hVR-1, hVR-2, and rVR-2 expression or activity.

Stimulation of hVR-1, hVR-2, and rVR-2 activity is desirable in situations in which hVR-1, hVR-2, and rVR-2 is abnormally downregulated and/or in which increased hVR-1, hVR-2, and rVR-2 activity is likely to have a beneficial effect. For
20 example, stimulation of hVR-1, hVR-2, and rVR-2 activity is desirable in situations in which an hVR-1, hVR-2, and rVR-2 is downregulated and/or in which increased hVR-1, hVR-2, and rVR-2 activity is likely to have a beneficial effect. Likewise, inhibition of hVR-1, hVR-2, and rVR-2 activity is desirable in situations in which hVR-1, hVR-2, and rVR-2 is abnormally upregulated and/or in which decreased hVR-1, hVR-2, and
25 rVR-2 activity is likely to have a beneficial effect.

3. Pharmacogenomics

The hVR-1, hVR-2, and rVR-2 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on hVR-1, hVR-2,
30 and rVR-2 activity (e.g., hVR-1, hVR-2, and rVR-2 gene expression) as identified by a screening assay described herein can be administered to individuals to treat

(prophylactically or therapeutically) hVR-1, hVR-2, and rVR-2-associated disorders (e.g., pain disorders) associated with aberrant hVR-1, hVR-2, and rVR-2 activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer an hVR-1, hVR-2, and rVR-2 molecule or hVR-1, hVR-2, and rVR-2 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with an hVR-1, hVR-2, and rVR-2 molecule or hVR-1, hVR-2, and rVR-2 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11):983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically

significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a

5 "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their

10 individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that

15 encodes a drugs target is known (*e.g.*, an hVR-1, hVR-2, and rVR-2 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a

20 major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug.

25 These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently

30 experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as

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demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

5 Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, an hVR-1, hVR-2, and rVR-2 molecule or hVR-1, hVR-2, and rVR-2 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

10 Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an hVR-1,
15 hVR-2, and rVR-2 molecule or hVR-1, hVR-2, and rVR-2 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

 This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent
20 applications cited throughout this application, as well as the Figures and the Sequence Listing are incorporated herein by reference.

EXAMPLES

25 **EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF hVR-1, hVR-2, and rVR-2 cDNA**

 In this example, the identification and characterization of the genes encoding hVR-1 (clone Fchrb87a6), hVR-2 (clone flh21e11), hVR-2 alternate form (clone frhob12c4), and rVR-2 (clone flrxb147g11) are described.

30

Isolation of the hVR-1, hVR-2, and the rVR-2 cDNA

The invention is based, at least in part, on the discovery of two human genes and one rat gene encoding novel members of the Capsaicin/Vanilloid receptor family, referred to herein as hVR-1, hVR-2, and rVR-2, respectively. These clones were identified from a human heart library and a rat dorsal root ganglion (DRG) library, based on sequence homology to the known rat VR-1 (Accession Number AF029310). The sequence of the two human clones and the rat clone was determined and found to contain open reading frames.

The nucleotide sequence of the full length hVR-1 cDNA and the predicted amino acid sequence of the hVR-1 polypeptide are shown in Figure 1 and in SEQ ID NOs:1 and 2, respectively.

The nucleotide sequence of the full length hVR-2 cDNA and the predicted amino acid sequence of the hVR-2 polypeptide are shown in Figure 2 and in SEQ ID NOs:4 and 5, respectively.

The nucleotide sequence of the partial hVR-2 (alternate form) cDNA and the predicted amino acid sequence of the hVR-2 (alternate form) polypeptide are shown in Figure 3 and in SEQ ID NOs:7 and 8, respectively.

The amino acid sequence of the predicted full length human VR-2 protein (alternate form) is shown in Figure 16 and in SEQ ID NO:20.

The nucleotide sequence of the partial rVR-2 cDNA and the predicted amino acid sequence of the rVR-2 polypeptide are shown in Figure 4 and in SEQ ID NOs:10 and 11, respectively.

Analysis of the hVR-1, hVR-2, and rVR-2 Molecules

The hVR-1 protein (SEQ ID NO:2) was aligned with the human VR-2 protein (SEQ ID NO:5) using the GAP program in the GCG software package (Blosom 62 matrix) and a gap weight of 12 and a length weight of 4. The results showed a 46.348% identity and 55.378% similarity between the two sequences (see Figure 5).

The hVR-1 nucleotide sequence (SEQ ID NO:1) was aligned with the human VR-2 nucleotide sequence (SEQ ID NO:4) using the GAP program in the GCG software package (nwsgapdna matrix) and a gap weight of 50 and a length weight of 3. The

results showed a 55.316% identity and 55.316% similarity between the two sequences (see Figure 6).

The hVR-2 protein (SEQ ID NO:5) was aligned with the rat VR-2 protein (SEQ ID NO:11) using the CLUSTAL W (1.74) multiple sequence alignment program (Figure 7), as well as using the GAP program in the GCG software package (Blosom 62 matrix) and a gap weight of 12 and a length weight of 4. The results showed a 79.167% identity and 81.703% similarity between the two sequences (see Figure 8).

The hVR-1 nucleotide sequence (SEQ ID NO:1) was aligned with the rat VR-1 nucleotide sequence (Accession Number:AF029310) using the GAP program in the GCG software package (nws gapdna matrix) and a gap weight of 50 and a length weight of 3. The results showed a 82.125% identity and 82.125% similarity between the two sequences (see Figure 9).

The hVR-1 protein (SEQ ID NO:2) was aligned with the rat VR-1 protein (Accession Number:AF029310) using the GAP program in the GCG software package (Blosom 62 matrix) and a gap weight of 12 and a length weight of 4. The results showed a 86.022% identity and 89.247% similarity between the two sequences (see Figure 10).

The hVR-2 protein (SEQ ID NO:5) was aligned with the human VR-2 protein (alternate form) (SEQ ID NO:8) using the CLUSTAL W (1.74) multiple sequence alignment program (Figure 11).

Finally, the hVR-2 protein (SEQ ID NO:5) was aligned with the predicted full length human VR-2 protein (alternate form) (SEQ ID NO:20) using the CLUSTAL W (1.74) multiple sequence alignment program (Figure 17).

A search was performed against the HMM database resulting in the identification of three ankyrin repeat domains in the amino acid sequence of hVR-1 (SEQ ID NO:2) at about residues 201-233, 248-283, and 333-361, and in the amino acid sequence of hVR-2 (SEQ ID NO:5) at about residues 162-194, 208-243, and 293-328. The results of the searches are set forth in Figures 13 and 15, respectively.

Hydropathy plots have identified 6 transmembrane domains in the hVR-1 and the hVR-2 proteins (see Figures 12 and 14, respectively).

A series of searches have revealed that the hVR-1 protein matches the ProDom entry 141801 for the vanilloid receptor subtype and the ProDom entry 145518 for the vanilloid receptor subtype.

Moreover, a search was performed against the Prosite database resulting in the
5 identification of four N-glycosylation sites in the amino acid sequence of SEQ ID NO:5 (at about residues 171-174, 192-195, 604-607, and 749-752), three cGMP-dependent protein kinase phosphorylation sites in the amino acid sequence of SEQ ID NO:5 (at about residues 2-5, 368-371, and 499-502), a series of protein kinase C and Casein kinase II phosphorylation sites in the amino acid sequence of SEQ ID NO:5, two
10 tyrosine kinase phosphorylation sites in the amino acid sequence of SEQ ID NO:5 (at about residues 368-375 and 622-628), and two myristoylation sites in the amino acid sequence of SEQ ID NO:5 (at about residues 169-174 and 765-770).

Tissue Distribution of hVR-1 and hVR-2 mRNA

15 This Example describes the tissue distribution of hVR-1 and hVR-2 mRNA as determined by *in situ* hybridization.

For *in situ* analysis, tissues, such as brain regions and whole brain, obtained from human and monkey were first frozen on dry ice. Ten-micrometer-thick coronal sections of the tissues were postfixed with 4% formaldehyde in DEPC treated 1X phosphate-
20 buffered saline at room temperature for 10 minutes before being rinsed twice in DEPC 1X phosphate-buffered saline and once in 0.1 M triethanolamine-HCl (pH 8.0). Following incubation in 0.25% acetic anhydride-0.1 M triethanolamine-HCl for 10 minutes, sections were rinsed in DEPC 2X SSC (1X SSC is 0.15M NaCl plus 0.015M sodium citrate). Tissue was then dehydrated through a series of ethanol washes,
25 incubated in 100% chloroform for 5 minutes, and then rinsed in 100% ethanol for 1 minute and 95% ethanol for 1 minute and allowed to air dry.

Hybridizations were performed with ³⁵S-radiolabeled (5 X 10⁷ cpm/ml) cRNA probes. Probes were incubated in the presence of a solution containing 600 mM NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 0.01% sheared salmon sperm DNA, 0.01% yeast
30 tRNA, 0.05% yeast total RNA type X1, 1 X Denhardt's solution, 50% formamide, 10%

dextran sulfate, 100 mM dithiothreitol, 0.1% sodium dodecyl sulfate (SDS), and 0.1% sodium thiosulfate for 18 hours at 55°C.

After hybridization, slides were washed with 2 X SSC. Sections were then sequentially incubated at 37°C in TNE (a solution containing 10 mM Tris-HCl (pH 7.6), 500 mM NaCl, and 1 mM EDTA), for 10 minutes, in TNE with 10µg of RNase A per ml for 30 minutes, and finally in TNE for 10 minutes. Slides were then rinsed with 2 X SSC at room temperature, washed with 2 X SSC at 50°C for 1 hour, washed with 0.2 X SSC at 55°C for 1 hour, and 0.2 X SSC at 60°C for 1 hour. Sections were then dehydrated rapidly through serial ethanol-0.3 M sodium acetate concentrations before being air dried and exposed to Kodak Biomax MR scientific imaging film for 24 hours and subsequently dipped in NB-2 photoemulsion and exposed at 4°C for 7 days before being developed and counter stained.

The data indicate that the hVR-1 molecule is not expressed in human nor monkey brain. The hVR-1 molecule is expressed in nodose, trigeminal sensory neurons, but is not expressed in sympathetic neurons. Within the nodose sensory neurons and trigeminal sensory neurons, expression was seen in distinct sub-populations. Moreover, hVR1 is expressed in some, but not all, small dorsal root ganglion (DRG) neurons and in a few medium sized DRG neurons. The hVR-1 molecule is partially co-expressed with the neuropeptide CGRP and with substance P which are present in nociceptive neurons.

The data further indicate that the VR-2 molecule is expressed in both human and monkey brain, primarily in cortical neurons. The VR2 molecule is also expressed in other brain regions, for example, the thalamus, striatum, hippocampus, hypothalamus, midbrain, medula and brain stem. In addition, the VR-2 molecule is expressed in parasympathetic neurons of the monkey heart (atrium), nodose sensory neurons, trigeminal (TRG) sensory neurons, dorsal root ganglion sensory neurons, sympathetic neurons, and motor neurons of the spinal cord. The VR2 molecule is widely expressed in TRG and DRG neurons, being present in most small and medium sized neurons and also in a few of the large neurons. VR2, like VR-1, partially co-localizes with CGRP and substance P.

Trigeminal sensory neurons are recognized pain centers while sympathetic neurons have been shown to be involved in neuropathic pain.

**EXAMPLE 2: EXPRESSION OF RECOMBINANT hVR-1, hVR-2, AND
rVR-2 PROTEIN IN BACTERIAL CELLS**

In this example, hVR-1, hVR-2, and rVR-2 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, hVR-1, hVR-2, and rVR-2 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-hVR-1, GST-hVR-2, and GST-rVR-2 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

**EXAMPLE 3: EXPRESSION OF RECOMBINANT hVR-1, hVR-2,
AND rVR-2 PROTEIN IN COS CELLS**

To express the hVR-1, hVR-2, and rVR-2 gene in COS cells, the pcDNA/Amp vector from Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire hVR-1, hVR-2, and rVR-2 protein and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the hVR-1, hVR-2, and rVR-2 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the hVR-1, hVR-2, and rVR-2 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop

codon, the HA tag or FLAG tag and the last 20 nucleotides of the hVR-1, hVR-2, and rVR-2 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two
5 restriction sites chosen are different so that the hVR-1, hVR-2, and rVR-2 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5a, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and
10 examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the hVR-1, hVR-2, and rVR-2-pCDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride coprecipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in
15 Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the hVR-1, hVR-2, and rVR-2 polypeptide is detected by radiolabelling (^{35}S -methionine or ^{35}S -cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D.
20 *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labelled for 8 hours with ^{35}S -methionine (or ^{35}S -cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture
25 media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the hVR-1, hVR-2, and rVR-2 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner
30 described above, and the expression of the hVR-1, hVR-2, and rVR-2 polypeptide is

detected by radiolabelling and immunoprecipitation using an hVR-1, hVR-2, and rVR-2 specific monoclonal antibody.

EXAMPLE 4: ELECTROPHYSIOLOGICAL STUDIES OF VR2

5 Human VR2 was functionally characterized in both HEK293 cells and *Xenopus* oocytes using electrophysiological methods. VR2 (in the pcDNA3.1 vector purchased by Invitrogen) was transiently expressed in HEK293 cells (ATCC) and recordings were performed 48 hours after transfection of cells using the whole-cell patch-clamp method (described in Bertil Hille, *Ionin Channels of excitable membranes*, 1992; Hammill *et al.* 10 (1981) *Pluger Arch.* 391:85-100). The results indicate that heat stimulation (>50 °C) induces a rapid inactivating inward current (1-2 nA). Heat-evoked currents of VR2 displayed profound desensitization and could be reversibly blocked by the VR1 inhibitor capsazepin (at a 10 µM concentration). In contrast to rat VR1, Capsaicin (at a 1-10 µM concentration), resiniferatoxin (at a 0.1-3 µM concentration), and low pH (5.0-6.0) do 15 not induce any currents from VR2. Binding studies of [³H]-resiniferatoxin (NEN) to both human VR1 and VR2 in membranes isolated from HEK293 cell homogenates also indicate that resiniferatoxin (at a 0.1-10 nM concentration) has no specific binding to VR2 while it binds to human VR1 with high affinities.

For the oocyte studies, human VR2 was subcloned into an oocyte expression 20 vector containing 5'- and 3'-UTR of *Xenopus* β-globin (Chiara *et al.* (1999) *Biochemistry* 38(20)6689-6698). *In vitro* trasncription was carried out as described in Chiara *et al.* (*supra*) and cRNA (10-100 ng) was then injected into the oocytes. VR2 function was characterized in the oocytes 48 hours after cRNA injection using a standard two-electrode voltage-clamp. Consistent with the data from the HEK293 studies, VR2 25 can only be activated by heat stimulation (48-50 °C) but not by vanilloid receptor agonists, capsaicin, or resiniferatoxin. The vanilloid receptor antagonist capsazepine (at a 1-10 µM concentration) blocks the heat response of VR2 reversibly.

EXAMPLE 5: GENERATION OF ANTI-hVR-2 ANTIBODIES AND hVR-2 PROTEIN LOCALIZATION BY IMMUNOSTAINING

Polyclonal antisera were raised in rabbits against the following three peptides derived from the human VR2 amino acid sequence, using the techniques described in Ed
5 Harlow and David Lane (1988) "Antibodies; A Laboratory Manual" Cold Spring harbor Laboratory Press.

Antibody PEPTIDE 1: AFHCKSPHRHRMVVLE (SEQ ID NO:13)

Antibody PEPTIDE 2: RPEAPTGNATESVQPMEGQEDEGN (SEQ ID NO:14)

Antibody PEPTIDE 3: SVLEMENGYWWCRKKQRAG (SEQ ID NO:15)

10 Antisera were subsequently affinity purified using the peptide immunogen.

The polyclonal antisera were tested for immunostaining of both monkey and rat dorsal root ganglion sensory neurons. Peptides 1 and 3 gave specific staining of subpopulations of sensory neurons that was competed with the corresponding peptide. This pattern of expression was very similar to the one observed using a VR-2 riboprobe.

15

EXAMPLE 6: CHROMOSOMAL LOCALIZATION OF hVR-1 AND hVR-2

To chromosomally map the hVR-1 gene, primers were designed based on the sequence of hVR-1 (clone Fchrb87a6) (amplifying a 177 bp product from a human
20 control cell line DNA and multiple faint larger products from a control Hamster cell line DNA by PCR). These primers were used to amplify 93 DNAs in duplicate from the Genebridge 4 Radiation Hybrid Panel (Research Genetics, Inc., Huntsville, AL).

The hVR-1 primers used in the PCR mapping studies were: forward - TAGGAGACCCCGTTGCCACG (SEQ ID NO:16) and reverse -
25 GATTCACCTGGGGACAGTGACG (SEQ ID NO:17) and the PCR reactions were performed as follows: 5 µl Template DNA (10ng/µl), 1.5µl 10X Perkin Elmer PCR Buffer, 1.2µl Pharmacia dNTP mix 2.5 mM, 1.15µl Forward primer 6.6µM, 1.15µl Reverse primer 6.6µM, 5µl Gibco/BRL Platinum Taq .05U/µl (Hot Start), using an amplification profile of: 95°C for 10 minutes followed by 35 Cycles of 94°C for 40
30 seconds, 55°C for 40 seconds, 72°C for 40 seconds, and 72°C for 5 minutes. The PCR

products were run on 2% agarose gels, post-stained with SYBR Gold (1:10,000 dilution in 1X TBE), and scanned on a Molecular Dynamics 595 Fluorimager.

The following is the vector data for the 93 Genebridge4 hybrid DNAs. These are in order 1-93. A "1" is a positive result, a "-" is a negative result, a "?" is an ambiguous result.

hVR1 1 - - 1 ? - 1 - 1 - 1 1 - - - 1 - - 1 - 1 1 - - 1 - 1 - 1 - - 1 1 - 1 - - - - - 1 - 1 1 1 - - -
1 - - - 1 1 - - - - 1 - - - - 1 - 1 - - 1 1 1 - 1 - 1 - - - 1 1 - 1 - - - - - 1

- 10 RH linkage analysis was performed using the Map Manager QTb28 software package.

hVR1 was found to map to the p arm of human chromosome 17, 18.9 cR₃₀₀₀ telomeric to the Whitehead Institute framework marker WI-6584, and 7.7 cR₃₀₀₀ centromeric of the Whitehead framework marker WI-5436. LOD scores for linkage were 14.5 for WI-6584 and 19.3 for WI-5436. This region corresponds to the
15 cytogenetic location 17p12-13. This region is syntenic to mouse chromosome 11.

To chromosomally map the hVR-2 gene, primers were designed from 5' UTR sequence of human VR2 (clone Flh21e11) (amplifying a 166 bp product from a human control cell line DNA and 2 much larger faint bands from a control Hamster cell line DNA by PCR). These primers were used to amplify 93 DNAs in duplicate from the
20 Genebridge 4 Radiation Hybrid Panel (Research Genetics, Inc., Huntsville, AL).

The hVR-2 primers used in the PCR mapping studies were: forward - TTAAGCTCCCGTTCTCACCG (SEQ ID NO:18) and reverse - GCTGCGGGAGGAAGTGAAGC (SEQ ID NO:19) and the PCR reactions were performed as follows: 5µl Template DNA (10ng/µl), 1.5µl 10X Perkin Elmer PCR
25 Buffer, 1.2µl Pharmacia dNTP mix 2.5mM, 1.15µl Forward primer 6.6µM, 1.15µl Reverse primer 6.6µM, 5µl Gibco/BRL Platinum Taq .05U/µl (Hot Start), using an amplification profile of 95°C for 10 minutes, followed by 35 Cycles of 94°C for 40 seconds, 55°C for 40 seconds, 72°C for 40 seconds, and 72°C for 5 minutes. The PCR
30 products were run on 2% agarose gels, post-stained with SYBR Gold (1:10,000 dilution in 1X TBE), and scanned on a Molecular Dynamics 595 Fluorimager.

The following is the vector data for the 93 Genebridge4 hybrid DNAs. These are in order 1-93. A "1" is a positive result, a "-" is a negative result, a "?" is an ambiguous result.

5 hVR2 1 - - 1 1 - ? 1 1 - - - - - 1 - - 1 - 1 1 - - - - 1 - 1 1 1 1 - - 1 - - - - - 1 1 - 1 1 1 - - 1
1 - - - 1 1 - - - - 1 1 1 - - - 1 - 1 1 - 1 - 1 1 1 1 1 - - - 1 1 - 1 - 1 - ? - - - ?

RH linkage analysis was performed using the Map Manager QTb28 software package.

10 hVR2 was found to map to the p arm of human chromosome 17 , 29.3cR cR₃₀₀₀ telomeric to the Whitehead Institute framework marker D17S721, and 23.3 cR₃₀₀₀ centromeric of the Whitehead framework marker AFMA043ZB5. LOD scores for linkage were 11.9 for D17S721 and 13.6 for AFMA043ZB5. This region corresponds to the cytogenetic location 17p11-12. This region is syntenic to mouse chromosome 11.

15

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

20 claims.

What is claimed:

1. An isolated nucleic acid molecule selected from the group consisting of:
 - (a) a nucleic acid molecule comprising the nucleotide sequence set
5 forth in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 or a complement thereof; and
 - (b) a nucleic acid molecule consisting of the nucleotide sequence set
forth in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 or a complement thereof.
- 10 2. An isolated nucleic acid molecule which encodes a polypeptide selected
from the group consisting of:
 - (a) a polypeptide comprising the amino acid sequence set forth in
SEQ ID NO:2, 5, 8, or 11; and
 - (b) a polypeptide consisting of the amino acid sequence set forth in
SEQ ID NO:2, 5, 8, or 11.
- 15 3. An isolated nucleic acid molecule which encodes a naturally occurring
allelic variant of a polypeptide comprising the amino acid sequence set forth in SEQ ID
NO:2, 5, 8, or 11.
- 20 4. An isolated nucleic acid molecule selected from the group consisting of:
 - a) a nucleic acid molecule comprising a nucleotide sequence which
is at least 83% identical to the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or
12, or a complement thereof;
 - b) a nucleic acid molecule comprising a fragment of at least 20
25 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, 3, 4,
6, 7, 9, 10, or 12, or a complement thereof;
 - c) a nucleic acid molecule which encodes a polypeptide comprising
an amino acid sequence at least about 87% identical to the amino acid sequence of SEQ
ID NO:2, 5, 8, or 11; and

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the fragment comprises at least 15 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2, 5, 8, or 11.

5

5. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1, 2, 3, or 4, and a nucleotide sequence encoding a heterologous polypeptide.

10

6. A vector comprising the nucleic acid molecule of any one of claims 1, 2, 3, or 4.

7. The vector of claim 6, which is an expression vector.

15

8. A host cell transfected with the expression vector of claim 7.

9. A method of expressing a polypeptide comprising culturing the host cell of claim 8 in an appropriate culture medium to, thereby, express the polypeptide.

10. An isolated polypeptide selected from the group consisting of:
- a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, 5, 8, or 11;
 - 5 b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule consisting of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 under stringent conditions;
 - c) a polypeptide which is encoded by a nucleic acid molecule
 - 10 comprising a nucleotide sequence which is at least 83% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12; and
 - d) a polypeptide comprising an amino acid sequence which is at least 87% identical to the amino acid sequence of SEQ ID NO:2, 5, 8, or 11.
- 15 11. The isolated polypeptide of claim 10 comprising the amino acid sequence of SEQ ID NO:2, 5, 8, or 11.
12. The polypeptide of claim 10, further comprising heterologous amino acid sequences.
- 20 13. An antibody which selectively binds to a polypeptide of claim 10.
14. A method for detecting the presence of a polypeptide of claim 10 in a sample comprising:
- 25 a) contacting the sample with a compound which selectively binds to the polypeptide; and
 - b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 10 in the sample.
- 30 15. The method of claim 14, wherein the compound which binds to the polypeptide is an antibody.

16. A kit comprising a compound which selectively binds to a polypeptide of claim 10 and instructions for use.

5 17. A method for detecting the presence of a nucleic acid molecule of any one of claims 1, 2, 3, or 4 in a sample comprising:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to a
10 nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of any one of claims 1, 2, 3, or 4 in the sample.

18. The method of claim 17, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

15

19. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of any one of claims 1, 2, 3, or 4 and instructions for use.

20. A method for identifying a compound which binds to a polypeptide of claim 10 comprising:

a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and

b) determining whether the polypeptide binds to the test compound.

25 21. The method of claim 20, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detection of test compound/polypeptide binding;

b) detection of binding using a competition binding assay; and

30 c) detection of binding using an assay for hVR-1, hVR-2, or rVR-2 activity.

22. A method for modulating the activity of a polypeptide of claim 10 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

5

23. A method for identifying a compound which modulates the activity of a polypeptide of claim 10 comprising:

- a) contacting a polypeptide of claim 10 with a test compound; and
 - b) determining the effect of the test compound on the activity of the
- 10 polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

24. A method for treating a subject having a disorder characterized by aberrant hVR-1 or hVR-2 protein activity or nucleic acid expression comprising

15 administering to the subject a hVR-1 or hVR-2 modulator such that treatment of the subject occurs.

25. The method of claim 24, wherein the hVR-1 or hVR-2 modulator is a small molecule.

20

26. The method of claim 24, wherein the disorder is a pain disorder.

1/35

humanVR1 gene with translation of open reading frame

Input file Fchrb87a6.seq; Output File Fchrb87a6.tra

Sequence length 3909

GTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAA
 CGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGG
 GCAGTGAGCGCAACGCACTTGCAGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGC
 GCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACT
 GCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTGCGGGCAGTG
 AGCGCAACGCACTGCGGGCAGTGAGCGCAACGCACTTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTTACAC
 TTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATT
 ACGCCAAGCTCTAATACGACTCACTATAGGGAAAGCTGGTACGCCTGCAGGTACCGGTCCGGAATTCCTGGGTGACACC
 ACGCGTCCGAAAACACACCTCTCTGCTGTGGGAAGACTGTGCAATGGCACAGCCGAGAGCTTGGTTTGGGAGGTTGAA
 GTGCTCTGGGGAGAATTCTAGATCATCCTCAGAAAAGCCTTGCCCTGGTGTCTACCAGAAAAACGCTCTCCCAATCAC
 CCAGAAAAGCTGTCCACAGTAGTCCCCCTTATCCACGGGTGTCACTTTCCATGGGTTCAAGTTATTTGCGGTCAACCAC
 GGTCTGCCAATATTAAATGGAATAATTCTTCAAACAGTTCCCAAGTTTCCCTTGTGCATTGTTCTGAGCAGTGTGATGA
 AGAGTCTCTGCCGTGCCATCTGGGATGCAAACCGTCCCTGTGTCCCCACGTCCAGGCCGTAGATGCTCCCCGCCGGTC
 AGTCACTTAGTCGTGAGATCGCCCGTCTGGTATCACAGTGCTTCTGTTTCAGGTTGCACACTGGGCCACAGAGGATCCA

 M K K W S S T D L G T A A D P L Q K 18
 GCAAGG ATG AAG AAA TGG AGC AGC ACA GAC TTG GGG ACA GCT GCG GAC CCA CTC CAA AAG 54

 D T C P D P L D G D P N S R P P P A K P 38
 GAC ACC TGC CCA GAC CCC CTG GAT GGA GAC CCT AAC TCC AGG CCA CCT CCA GCC AAG CCC 114

 Q L P T A K S R T R L F G K G D S E E A 58
 CAG CTC CCC ACG GCC AAG AGC CGC ACC CGG CTC TTT GGG AAG GGT GAC TCG GAG GAG GCT 174

 F P V D C P H E E G E L D S C P T I T V 78
 TTC CCG GTG GAT TGC CCC CAC GAG GAA GGT GAG TTG GAC TCC TGC CCG ACC ATC ACA GTC 234

 S P V I T I Q R P G D G P T G A R L L S 98
 AGC CCT GTT ATC ACC ATC CAG AGG CCA GGA GAC GGC CCC ACC GGT GCC AGG CTG CTG TCC 294

 Q D S V A A S T E K T L R L Y D R R S I 118
 CAG GAC TCT GTC GCC GCC AGC ACC GAG AAG ACC CTC AGG CTC TAT GAT CGC AGG AGT ATC 354

 F E A V A Q N N C Q D L E S L L L F L Q 138
 TTT GAA GCC GTT GCT CAG AAT AAC TGC CAG GAT CTG GAG AGC CTG CTG CTC TTC CTG CAG 414

FIGURE 1

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K	S	K	K	H	L	T	D	N	E	F	K	D	P	E	T	G	K	T	C	158
AAG	AGC	AAG	AAG	CAC	CTC	ACA	GAC	AAC	GAG	TTC	AAA	GAC	CCT	GAG	ACA	GGG	AAG	ACC	TGT	474
L	L	K	A	M	L	N	L	H	D	G	Q	N	T	T	I	P	L	L	L	178
CTG	CTG	AAA	GCC	ATG	CTC	AAC	CTG	CAC	GAC	GGA	CAG	AAC	ACC	ACC	ATC	CCC	CTG	CTC	CTG	534
E	I	A	R	Q	T	D	S	L	K	E	L	V	N	A	S	Y	T	D	S	198
GAG	ATC	GCG	CGG	CAA	ACG	GAC	AGC	CTG	AAG	GAG	CTT	GTC	AAC	GCC	AGC	TAC	ACG	GAC	AGC	594
Y	Y	K	G	Q	T	A	L	H	I	A	I	E	R	R	N	M	A	L	V	218
TAC	TAC	AAG	GGC	CAG	ACA	GCA	CTG	CAC	ATC	GCC	ATC	GAG	AGA	CGC	AAC	ATG	GCC	CTG	GTG	654
T	L	L	V	E	N	G	A	D	V	Q	A	A	A	H	G	D	F	F	K	238
ACC	CTC	CTG	GTG	GAG	AAC	GGA	GCA	GAC	GTC	CAG	GCT	GCG	GCC	CAT	GGG	GAC	TTC	TTT	AAG	714
K	T	K	G	R	P	G	F	Y	F	G	E	L	P	L	S	L	A	A	C	258
AAA	ACC	AAA	GGG	CGG	CCT	GGA	TTC	TAC	TTC	GGT	GAA	CTG	CCC	CTG	TCC	CTG	GCC	GCG	TGC	774
T	N	Q	L	G	I	V	K	F	L	L	Q	N	S	W	Q	T	A	D	I	278
ACC	AAC	CAG	CTG	GGC	ATC	GTG	AAG	TTC	CTG	CTG	CAG	AAC	TCC	TGG	CAG	ACG	GCC	GAC	ATC	834
S	A	R	D	S	V	G	N	T	V	L	H	A	L	V	E	V	A	D	N	298
AGC	GCC	AGG	GAC	TCG	GTG	GGC	AAC	ACG	GTG	CTG	CAC	GCC	CTG	GTG	GAG	GTG	GCC	GAC	AAC	894
T	A	D	N	T	K	F	V	T	S	M	Y	N	E	I	L	M	L	G	A	318
ACG	GCC	GAC	AAC	ACG	AAG	TTT	GTG	ACG	AGC	ATG	TAC	AAT	GAG	ATT	CTG	ATG	CTG	GGG	GCC	954
K	L	H	P	T	L	K	L	E	E	L	T	N	K	K	G	M	T	P	L	338
AAA	CTG	CAC	CCG	ACG	CTG	AAG	CTG	GAG	GAG	CTC	ACC	AAC	AAG	AAG	GGA	ATG	ACG	CCG	CTG	1014
A	L	A	A	G	T	G	K	I	G	V	L	A	Y	I	L	Q	R	E	I	358
GCT	CTG	GCA	GCT	GGG	ACC	GGG	AAG	ATC	GGG	GTC	TTG	GCC	TAT	ATT	CTC	CAG	CGG	GAG	ATC	1074
Q	E	P	E	C	R	H	L	S	R	K	F	T	E	W	A	Y	G	P	V	378
CAG	GAG	CCC	GAG	TGC	AGG	CAC	CTG	TCC	AGG	AAG	TTC	ACC	GAG	TGG	GCC	TAC	GGG	CCC	GTG	1134
H	S	S	L	Y	D	L	S	C	I	D	T	C	E	K	N	S	V	L	E	398
CAC	TCC	TCG	CTG	TAC	GAC	CTG	TCC	TGC	ATC	GAC	ACC	TGC	GAG	AAG	AAC	TCG	GTG	CTG	GAG	1194
V	I	A	Y	S	S	S	E	T	P	N	R	H	D	M	L	L	V	E	P	418
GTG	ATC	GCC	TAC	AGC	AGC	AGC	GAG	ACC	CCT	AAT	CGC	CAC	GAC	ATG	CTC	TTG	GTG	GAG	CCG	1254
L	N	R	L	L	Q	D	K	W	D	R	F	V	K	R	I	F	Y	F	N	438
CTG	AAC	CGA	CTC	CTG	CAG	GAC	AAG	TGG	GAC	AGA	TTC	GTC	AAG	CGC	ATC	TTC	TAC	TTC	AAC	1314
F	L	V	Y	C	L	Y	M	I	I	F	T	M	A	A	Y	Y	R	P	V	458
TTC	CTG	GTC	TAC	TGC	CTG	TAC	ATG	ATC	ATC	TTC	ACC	ATG	GCT	GCC	TAC	TAC	AGG	CCC	GTG	1374
D	G	L	P	P	F	K	M	E	K	I	G	D	Y	F	R	V	T	G	E	478
GAT	GGC	TTG	CCT	CCC	TTT	AAG	ATG	GAA	AAA	ATT	GGA	GAC	TAT	TTC	CGA	GTT	ACT	GGA	GAG	1434
I	L	S	V	L	G	G	V	Y	F	F	F	R	G	I	Q	Y	F	L	Q	498
ATC	CTG	TCT	GTG	TTA	GGA	GGA	GTC	TAC	TTC	TTT	TTC	CGA	GGG	ATT	CAG	TAT	TTC	CTG	CAG	1494

FIGURE 1 (cont'd)

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R	R	P	S	M	K	T	L	F	V	D	S	Y	S	E	M	L	F	F	L	518
AGG	CGG	CCG	TCG	ATG	AAG	ACC	CTG	TTT	GTG	GAC	AGC	TAC	AGT	GAG	ATG	CTT	TTC	TTT	CTG	1554
Q	S	L	F	M	L	A	T	V	V	L	Y	F	S	H	L	K	E	Y	V	538
CAG	TCA	CTG	TTC	ATG	CTG	GCC	ACC	GTG	GTG	CTG	TAC	TTC	AGC	CAC	CTC	AAG	GAG	TAT	GTG	1614
A	S	M	V	F	S	L	A	L	G	W	T	N	M	L	Y	Y	T	R	G	558
GCT	TCC	ATG	GTA	TTC	TCC	CTG	GCC	TTG	GGC	TGG	ACC	AAC	ATG	CTC	TAC	TAC	ACC	CGC	GGT	1674
F	Q	Q	M	G	I	Y	A	V	M	I	E	K	M	I	L	R	D	L	C	578
TTC	CAG	CAG	ATG	GGC	ATC	TAT	GCC	GTC	ATG	ATA	GAG	AAG	ATG	ATC	CTG	AGA	GAC	CTG	TGC	1734
R	F	M	F	V	Y	I	V	F	L	F	G	F	S	T	A	V	V	T	L	598
CGT	TTC	ATG	TTT	GTC	TAC	ATC	GTC	TTC	TTG	TTC	GGG	TTT	TCC	ACA	GCG	GTG	GTG	ACG	CTG	1794
I	E	D	G	K	N	D	S	L	P	S	E	S	T	S	H	R	W	R	G	618
ATT	GAA	GAC	GGG	AAG	AAT	GAC	TCC	CTG	CCG	TCT	GAG	TCC	ACG	TCG	CAC	AGG	TGG	CGG	GGG	1854
P	A	C	R	P	P	D	S	S	Y	N	S	L	Y	S	T	C	L	E	L	638
CCT	GCC	TGC	AGG	CCC	CCC	GAT	AGC	TCC	TAC	AAC	AGC	CTG	TAC	TCC	ACC	TGC	CTG	GAG	CTG	1914
F	K	F	T	I	G	M	G	D	L	E	F	T	E	N	Y	D	F	K	A	658
TTC	AAG	TTC	ACC	ATC	GGC	ATG	GGC	GAC	CTG	GAG	TTC	ACT	GAG	AAC	TAT	GAC	TTC	AAG	GCT	1974
V	F	I	I	L	L	L	A	Y	V	I	L	T	Y	I	L	L	L	N	M	678
GTC	TTC	ATC	ATC	CTG	CTG	CTG	GCC	TAT	GTA	ATT	CTC	ACC	TAC	ATC	CTC	CTG	CTC	AAC	ATG	2034
L	I	A	L	M	G	E	T	V	N	K	I	A	Q	E	S	K	N	I	W	698
CTC	ATC	GCC	CTC	ATG	GGT	GAG	ACT	GTC	AAC	AAG	ATC	GCA	CAG	GAG	AGC	AAG	AAC	ATC	TGG	2094
K	L	Q	R	A	I	T	I	L	D	T	E	K	S	F	L	K	C	M	R	718
AAG	CTG	CAG	AGA	GCC	ATC	ACC	ATC	CTG	GAC	ACG	GAG	AAG	AGC	TTC	CTT	AAG	TGC	ATG	AGG	2154
K	A	F	R	S	G	K	L	L	Q	V	G	Y	T	P	D	G	K	D	D	738
AAG	GCC	TTC	CGC	TCA	GGC	AAG	CTG	CTG	CAG	GTG	GGG	TAC	ACA	CCT	GAT	GGC	AAG	GAC	GAC	2214
Y	R	W	C	F	R	V	D	E	V	N	W	T	T	W	N	T	N	V	G	758
TAC	CGG	TGG	TGC	TTC	AGG	GTG	GAC	GAG	GTG	AAC	TGG	ACC	ACC	TGG	AAC	ACC	AAC	GTG	GGC	2274
I	I	N	E	D	P	G	N	C	E	G	V	K	R	T	L	S	F	S	L	778
ATC	ATC	AAC	GAA	GAC	CCG	GGC	AAC	TGT	GAG	GGC	GTC	AAG	CGC	ACC	CTG	AGC	TTC	TCC	CTG	2334
R	S	S	R	V	S	G	R	H	W	K	N	F	A	L	V	P	L	L	R	798
CGG	TCA	AGC	AGA	GTT	TCA	GGC	AGA	CAC	TGG	AAG	AAC	TTT	GCC	CTG	GTC	CCC	CTT	TTA	AGA	2394
E	A	S	A	R	D	R	Q	S	A	Q	P	E	E	V	Y	L	R	Q	F	818
GAG	GCA	AGT	GCT	CGA	GAT	AGG	CAG	TCT	GCT	CAG	CCC	GAG	GAA	GTT	TAT	CTG	CGA	CAG	TTT	2454
S	G	S	L	K	P	E	D	A	E	V	F	K	S	P	A	A	S	G	E	838
TCA	GGG	TCT	CTG	AAG	CCA	GAG	GAC	GCT	GAG	GTC	TTC	AAG	AGT	CCT	GCC	GCT	TCC	GGG	GAG	2514
K	*																			840
AAG	TGA																			2520

FIGURE 1 (cont'd)

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GGACGTCACGCAGACAGCACTGTCAACACTGGGCCCTTAGGAGACCCCGTTGCCACGGGGGGCTGCTGAGGGAAACACCAG
TGCTCTGTTCAGCAGCCCTGGCCCTGGTCTGTGCGCTGCCCAGGCATGTTCCCAAATCTGTGCTGGACAAGCTGTGGGAAGCGT
TCTTGGAGCATGGGGAGTGATGTACATCCAAACCGTCACTGTCTCCCAAGTGAATCTCCTAACAGACTTTTCAGGTTTTTA
CTCACTTTACTAAAAAAGGGCGCGCTTA

FIGURE 1 (cont'd)

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Full-length human VR2

Input file Flh2le11.seq; Output File Flh2le11.tra
Sequence length 2809

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GGCTAGCCTGTCTGACAGGGGAGAGTTAAGCTCCCGTTCTCCACCGTGCCGGCTGGCCAGGTGGGCTGAGGGTGACCG
AGAGACCAGAACCTGCTTGCTGGAGCTTAGTGCTCAGAGCTGGGGAGGGAGGTTCCGCGCTCCTCTGCTGTCAGCGCC
GGCAGCCCCCTCCCGGCTTCACTTCCCTCCCGCAGCCCCTGCTACTGAGAAGCTCCGGGATCCAGCAGCCGCCACGCCCT
GGCCTCAGCCTGCGGGGCTCCAGTCAGGCCAACACCGACGCGCAGCTGGGAGGAAGACAGGACCCCTTGACATCTCCATC

                                M T S P S S S P      8
TGACAGAGGTCTGGCTGGACCGAGCAGCCTCCTCCTCTAGG ATG ACC TCA CCC TCC AGC TCT CCA      24

V F R L E T L D G G Q E D G S E A D R G      28
GTT TTC AGG TTG GAG ACA TTA GAT GGA GGC CAA GAA GAT GGC TCT GAG GCG GAC AGA GGA      84

K L D F G S G L P P M E S Q F Q G E D R      48
AAG CTG GAT TTT GGG AGC GGG CTG CCT CCC ATG GAG TCA CAG TTC CAG GGC GAG GAC CGG      144

K F A P Q I R V N L N Y R K G T G A S Q      68
AAA TTC GCC CCT CAG ATA AGA GTC AAC CTC AAC TAC CGA AAG GGA ACA GGT GCC AGT CAG      204

P D P N R F D R D R L F N A V S R G V P      88
COG GAT CCA AAC CGA TTT GAC CGA GAT CGG CTC TTC AAT GCG GTC TCC CGG GGT GTC CCC      264

E D L A G L P E Y L S K T S K Y L T D S      108
GAG GAT CTG GCT GGA CTT CCA GAG TAC CTG AGC AAG ACC AGC AAG TAC CTC ACC GAC TOG      324

E Y T E G S T G K T C L M K A V L N L K      128
GAA TAC ACA GAG GGC TCC ACA GGT AAG ACG TGC CTG ATG AAG GCT GTG CTG AAC CTT AAG      384

D G V N A C I L P L L Q I D R D S G N P      148
GAC GGA GTC AAT GCC TGC ATT CTG CCA CTG CTG CAG ATC GAC AGG GAC TCT GGC AAT CCT      444

Q P L V N A Q C T D D Y Y R G H S A L H      168
CAG CCC CTG GTA AAT GCC CAG TGC ACA GAT GAC TAT TAC CGA GGC CAC AGC GCT CTG CAC      504

I A I E K R S L Q C V K L L V E N G A N      188
ATC GCC ATT GAG AAG AGG AGT CTG CAG TGT GTG AAG CTC CTG GTG GAG AAT GGG GCC AAT      564

V H A R A C G R F F Q K G Q G T C F Y F      208
GTG CAT GCC CGG GGC TGC GGC CGC TTC TTC CAG AAG GGC CAA GGG ACT TGC TTT TAT TTC      624

G E L P L S L A A C T K Q W D V V S Y L      228
GGT GAG CTA CCC CTC TCT TTG GCC GCT TGC ACC AAG CAG TGG GAT GTG GTA AGC TAC CTC      684

L E N P H Q P A S L Q A T D S Q G N T V      248
CTG GAG AAC CCA CAC CAG CCC GGC AGC CTG CAG GGC ACT GAC TCC CAG GGC AAC ACA GTC      744

L H A L V M I S D N S A E N I A L V T S      268
CTG CAT GCC CTA GTG ATG ATC TOG GAC AAC TCA GCT GAG AAC ATT GCA CTG GTG ACC AGC      804

M Y D G L L Q A G A R L C P T V Q L E D      288
ATG TAT GAT GGG CTC CTC CAA GCT GGG GCC CGC CTC TGC CCT ACC GTG CAG CTT GAG GAC      864

I R N L Q D L T P L K L A A K E G K I E      308

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FIGURE 2

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ATC CGC AAC CTG CAG GAT CTC ACG CCT CTG AAG CTG GCC GCC AAG GAG GGC AAG ATC GAG	924
I F R H I L Q R E F S G L S H L S R K F	328
ATT TTC AGG CAC ATC CTG CAG CGG GAG TTT TCA GGA CTG AGC CAC CTT TCC CGA AAG TTC	984
T E W C Y G P V R V S L Y D L A S V D S	348
ACC GAG TGG TGC TAT GGG CCT GTC CGG GTG TCG CTG TAT GAC CTG GCT TCT GTG GAC AGC	1044
C E E N S V L E I I A F H C K S P H R H	368
TGT GAG GAG AAC TCA GTG CTG GAG ATC ATT GCC TTT CAT TGC AAG AGC CGG CAC CGA CAC	1104
R M V V L E P L N K L L Q A K W D L L I	388
CGA ATG GTC GTT TTG GAG CCC CTG AAC AAA CTG CTG CAG GCG AAA TGG GAT CTG CTC ATC	1164
P K F F L N F L C N L I Y M F I F T A V	408
CCC AAG TTC TTC TTA AAC TTC CTG TGT AAT CTG ATC TAC ATG TTC ATC TTC ACC GCT GTT	1224
A Y H Q P T L K K Q A A P H L K A E V G	428
GCC TAC CAT CAG CCT ACC CTG AAG AAG CAG GCC GCC CCT CAC CTG AAA GCG GAG GTT GGA	1284
N S M L L T G H I L I L L G G I Y L L V	448
AAC TCC ATG CTG CTG ACG GGC CAC ATC CTT ATC CTG CTA GGG GGG ATC TAC CTC CTC GTG	1344
G Q L W Y F W R R H V F I W I S F I D S	468
GGC CAG CTG TGG TAC TTC TGG CGG CGC CAC GTG TTC ATC TGG ATC TCG TTC ATA GAC AGC	1404
Y F E I L F L F Q A L L T V V S Q V L C	488
TAC TTT GAA ATC CTC TTC CTG TTC CAG GCC CTG CTC ACA GTG GTG TCC CAG GTG CTG TGT	1464
F L A I E W Y L P L L V S A L V L G W L	508
TTC CTG GCC ATC GAG TGG TAC CTG CCC CTG CTT GTG TCT GCG CTG GTG CTG GGC TGG CTG	1524
N L L Y Y T R G F Q H T G I Y S V M I Q	528
AAC CTG CTT TAC TAT ACA CGT GGC TTC CAG CAC ACA GGC ATC TAC AGT GTC ATG ATC CAG	1584
K V I L R D L L R F L L I Y L V F L F G	548
AAG GTC ATC CTG CGG GAC CTG CGC TTC CTT CTG ATC TAC TTA GTC TTC CTT TTC GGC	1644
F A V A L V S L S Q E A W R P E A P T G	568
TTC GCT GTA GCC CTG GTG AGC CTG AGC CAG GAG GCT TGG CGC CCC GAA GCT CCT ACA GGC	1704
P N A T E S V Q P M E G Q E D E G N G A	588
CCC AAT GCC ACA GAG TCA GTG CAG CCC ATG GAG GGA CAG GAG GAC GAG GGC AAC GGC GCC	1764
Q Y R G I L E A S L E L F K F T I G M G	608
CAG TAC AAG GGT ATC CTG GAA GCC TCC TTG GAG CTC TTC AAA TTC ACC ATC GGC ATG GGC	1824
E L A F Q E Q L E F R G M V L L L L L A	628
GAG CTG GCC TTC CAG GAG CAG CTG CAC TTC CGC GGC ATG GTG CTG CTG CTG CTG GGC	1884
Y V L L T Y I L L L N H L I A L M S E T	648
TAC GTG CTG CTC ACC TAC ATC CTG CTG CTC AAC ATG CTC ATC GCC CTC ATG AGC GAG ACC	1944
V N S V A T D S W S I W K L Q K A I S V	668
GTC AAC AGT GTC GCC ACT GAC AGC TGG AGC ATC TGG AAG CTG CAG AAA GCC ATC TCT GTC	2004
L E H E N G Y W W C R K K Q R A G V M L	688
CTG GAG ATG GAG AAT GGC TAT TGG TGG TGC AAG AAG AAG CAG CGG GCA GGT GTG ATG CTG	2064
T V G T K P D G S P D E R W C F R V E E	708

FIGURE 2 (continued)

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ACC GTT GGC ACT AAG CCA GAT GGC AGC CCG GAT GAG CGC TGG TGC TTC AGG GTG GAG GAG 2124
V N W A S W E Q T L P T L C E D P S G A 728
GTG AAC TGG GCT TCA TGG GAG CAG ACG CTG CCT ACG CTG TGT GAG GAC CCG TCA GGG GCA 2184
G V P R T L E N P V L A S P P K E D E D 748
GGT GTC CCT CGA ACT CTC GAG AAC CCT GTC CTG GCT TCC CCT CCC AAG GAG GAT GAG GAT 2244
G A S E E N Y V P V Q L L Q S N * 765
GGT GCC TCT GAG GAA AAC TAT GTG CCC GTC CAG CTC CAG TCC AAC TGA 2295
TGGCCAGATGCAGCAGGCGCAGAGCAGAGCAGAGGATCTTTCCAAACCACATCTGCTGGCTCTGGGGTCCCGTG
AATTCTGGTGGCAATATATATTTTCACTAACTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIGURE 2 (continued)

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Partial human VR2 alternate form

Input file frhob12c4.seq; Output File frhob12c4.tra
Sequence length 1489

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      G R F F Q K G Q G T C F Y F G E L P L      19
GC GGC CGC TTC TTC CAG AAG GGC CAA GGG ACT TGC TTT TAT TTC GGT GAG CTA CCC CTC      57

      S L A A C T K Q W D V V S Y L L E N P H      39
TCT TTG GCC GCT TGC ACC AAG CAG TGG GAT GTG GTA AGC TAC CTC CTG GAG AAC CCA CAC      117

      Q P A S L Q A T D S Q G N T V L H A L V      59
CAG CCC GCC AGC CTG CAG GCC ACT GAC TCC CAG GGC AAC ACA GTC CTG CAT GCC CTA GTG      177

      M I S D N S A E N I A L V T S M Y D G L      79
ATG ATC TCG GAC AAC TCA GCT GAG AAC ATT GCA CTG GTG ACC AGC ATG TAT GAT GGG CTC      237

      L Q A G A R L C P T V Q L E D I R N L Q      99
CTC CAA GCT GGG GCC CGC CTC TGC CCT ACC GTG CAG CTT GAG GAC ATC CGC AAC CTG CAG      297

      D L T P L K L A A K E G K I E I F R H I      119
GAT CTC ACG CCT CTG AAG CTG GCC GCC AAG GAG GGC AAG ATC GAG ATT TTC AGG CAC ATC      357

      L Q R E F S G L S H L S R K P T E W C Y      139
CTG CAG CGG GAG TTT TCA GGA CTG AGC CAC CTT TCC CGA AAG TTC ACC GAG TGG TGC TAT      417

      G P V R V S L Y D L A S V D S C E E N S      159
GGG CCT GTC CGG GTG TCG CTG TAT GAC CTG GCT TCT GTG GAC AGC TGT GAG GAG AAC TCA      477

      V L E I I A F H C K S P H R H R M V V L      179
GTG CTG GAG ATC ATT GCC TTT CAT TGC AAG AGC CGG CAC CGA CAC CGA ATG GTC GTT TTG      537

      E P L N K L L Q A K N D L L I P K F F L      199
GAG CCC CTG AAC AAA CTG CTG CAG GCG AAA TGG GAT CTG CTC ATC CCC AAG TTC TTC TTA      597

      N F L C N L I Y M F I F T A V A Y H Q P      219
AAC TTC CTG TGT AAT CTG ATC TAC ATG TTC ATC TTC ACC GCT GTT GCC TAC CAT CAG CCT      657

      T L K K Q A A P H L K A E V G N S M L L      239
ACC CTG AAG AAG CAG GCC GCC CCT CAC CTG AAA GCG GAG GTT GGA AAC TCC ATG CTG CTG      717

      T G H I L I L L G G I Y L L V G Q L W Y      259
ACG GGC CAC ATC CTT ATC CTG CTA GGG GGG ATC TAC CTC CTC GTG GGC CAG CTG TGG TAC      777

      F W R R H V F I W I S F I D S Y F E I L      279
TTC TGG CGG CGC CAC GTG TTC ATC TGG ATC TGG TTC ATA GAC AGC TAC TTT GAA ATC CTC      837

      F L F Q A L L T V V S Q V L C F L A I E      299
TTC CTG TTC CAG GCC CTG CTC ACA GTG GTG TCC CAG GTG CTG TGT TTC CTG GCC ATC GAG      897

      W Y L P L L V S A L V L G W L N L L Y Y      319
TGG TAC CTG CCC CTG CTT GTG TCT GCG CTG GTG CTG GGC TGG CTG AAC CTG CTT TAC TAT      957

      T R G F Q H T G I Y S V M I Q K K A I S      339
ACA CGT GGC TTC CAG CAC ACA GGC ATC TAC AGT GTC ATG ATC CAG AAG AAA GCC ATC TCT      1017

      V L E H E H G Y W W C R K K Q R A G V M      359
GTC CTG GAG ATG GAG AAT GGC TAT TGG TGG TGC AGG AAG AAG CAG CGG GCA GGT GTG ATG      1077

```

FIGURE 3

L T V G T K P D G S P D E R W C F R V E 379
CTG ACC GTT GGC ACT AAG CCA GAT GGC AGC CCG GAT GAG CGC TGG TGC TTC AGG GTG GAG 1137

E V N W A S W E Q T L P T L C E D P S G 399
GAG GTG AAC TGG GCT TCA TGG GAG CAG ACG CTG CCT ACG CTG TGT GAG GAC CCG TCA GGG 1197

A G V P R T L E N P V L A S P P K E D E 419
GCA GGT GTC CCT CGA ACT CTC GAG AAC CCT GTC CTG GCT TCC CCT CCC AAG GAG GAT GAG 1257

D G A S E E N Y V P V Q L L Q S N * 437
GAT GGT GCC TCT GAG GAA AAC TAT GTG CCC GTC CAG CTC CAG TCC AAC TGA 1311

TGGCCAGATGCGAGGAGGCGCCAGAGCAGAGCAGAGGATCTTTCCAAACCACATCTGCTGGCTCTGGGGTCCAGTG
AATTCTGGTGGCAATATATATATTTTCATCTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGA
GCGGACCGGTGGGTGGAC

FIGURE 3 (continued)

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Partial rat VR2

Input file Flrxb147g11.seq; Output File Flrxb147g11.trc
Sequence length 1794

S T H A S A L S L A A C T K Q W D V V	19
G TCG ACC CAC GCG TCC GCT CTT TCT CTG GCT GCG TGC ACC AAG CAG TGG GAT GTG GTG	57
T Y L L E N P H Q P A S L E A T D S L G	39
ACC TAC CTC CTG GAG AAC CCA CAC CAG CCG GCC AGC CTG GAG GCC ACC GAC TCC CTG GGC	117
N T V L H A L V M I A D N S P E N S A L	59
AAC ACA GTC CTG CAT GCT CTG GTA ATG ATT GCA GAT AAC TCG CCT GAG AAC AGT GCC CTG	177
V I H M Y D G L L Q M G A R L C P T V Q	79
GTG ATC CAC ATG TAC GAC GGG CTT CTA CAA ATG GGG GCG CGC CTC TGC CCC ACT GTG CAG	237
L E E I S N H Q G L T P L K L A A K E G	99
CTT GAG GAA ATC TCC AAC CAC CAA GGC CTC ACA CCC CTG AAA CTA GCC GCC AAG GAA GGC	297
K I E I F R H I L Q R E F S G P Y Q P L	119
AAA ATC GAG ATT TTC AGG CAC ATT CTG CAG CGG GAA TTC TCA GGA CCG TAC CAG CCC CTT	357
S R K F T E W C Y G P V R V S L Y D L S	139
TCC CGA AAG TTT ACT GAG TGG TGT TAC GGT CCT GTG CGG GTA TCG CTG TAC GAC CTG TCC	417
S V D S W E K N S V L E I I A F H C K S	159
TCT GTG GAC AGC TGG GAA AAG AAC TCG GTG CTG GAG ATC ATC GCT TTT CAT TGC AAG AGC	477
P N R H R M V V L E P L N K L L Q E K W	179
CCG AAC CGG CAC CGC ATG GTG GTT TTA GAA CCA CTG AAC AAG CTT CTG CAG GAG AAA TGG	537
D R L V S R F F F N F A C Y L V Y M F I	199
GAT CGG CTC GTC TCA AGA TTC TTC AAC TTC GCC TGC TAC TTG GTC TAC ATG TTC ATC	597
F T V V A Y H Q P S L D Q P A I P S S K	219
TTC ACC GTC GTT GCC TAC CAC CAG CCT TCC CTG GAT CAG CCA GCC ATC CCC TCA TCA AAA	657
A T F G E S M L L L G H I L I L L G G I	239
GCG ACT TTT GGG GAA TCC ATG CTG CTG CTG GGC CAC ATT CTG ATC CTG CTT GGG GGT ATT	717
Y L L L G Q L W Y F W R R R L F I W I S	259
TAC CTC TTA CTG GGC CAG CTG TGG TAC TTT TGG CGG CGG CGC CTG TTT ATC TGG ATC TCA	777
F M D S Y F E I L F L L Q A L L T V L S	279
TTC ATG GAC AGC TAC TTT GAA ATC CTC TTT CTC CTT CAG GCT CTG CTC ACA GTG CTG TCC	837
Q V L R F M E T E W Y L P L L V L S L V	299
CAG GTG CTG CGC TTC ATG GAG ACT GAA TGG TAC CTA CCC CTG CTA GTG TTA TCC CTA GTG	897
L G W L N L L Y Y T R G F Q H T G I Y S	319
CTG GGC TGG CTG AAC CTG CTT TAC TAC ACA CGG GGC TTT CAG CAC ACA GGC ATC TAC AGT	957
V M I Q K V I L R D L L R F L L V Y L V	339
GTC ATG ATC CAG AAG GTC ATC CTT CGA GAC CTG CTC CGT TTC CTG CTG GTC TAC CTG GTC	1017
F L F G F A V A L V S L S R E A R S P K	359
TTC CTT TTC GGC TTT GCT GTA GCC CTA GTA AGC TTG AGC AGA GAG GGC CGA AGT CCC AAA	1077

FIGURE 4

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A P E D N N S T V T E Q P T V G Q E E E 379
 GCC CCT GAA GAT AAC AAC TCC ACA GTG ACG GAA CAG CCC ACG GTG GGC CAG GAG GAG GAG 1137

 P A P Y R S I L D A S L E L F K F T I G 399
 CCA GCT CCA TAT CCG AGC ATT CTG GAT GCC TCC CTA GAG CTG TTC AAG TTC ACC ATT GGT 1197

 M G E L A F Q E Q L R F R G V V L L L L 419
 ATG GGG GAG CTG GCT TTC CAG GAA CAG CTG CGT TTT CGT GGG GTG GTC CTG CTG TTG CTG 1257

 L A Y V L L T Y V L L L L N M L I A L M S 439
 TTG GCC TAC GTC CTT CTC ACC TAC GTC CTG CTC AAC ATG CTC ATT GCT CTC ATG AGC 1317

 E T V N H V A D N S W S I W K L Q K A I 459
 GAA ACT GTC AAC CAC GTT GCT GAC AAC AGC TGG AGC ATC TGG AAG TTG CAG AAA GCC ATC 1377

 S V L E M E N G Y W W C R R K K H R E G 479
 TCT GTC TTG GAG ATG GAG AAT GGT TAC TGG TGG TGC CGG AGG AAG AAA CAT CGT GAA GGG 1437

 R L L K V G T R G D G T P D E R W C F R 499
 AGG CTG CTG AAA GTC GGC ACC AGG GGG GAT GGT ACC CCT GAT GAG CGC TGG TGC TTC AGG 1497

 V E E V N W A A W E K T L P T L S E D P 519
 GTG GAG GAA GTA AAT TGG GCT GCT TGG GAG AAG ACT CTT CCC ACC TTA TCT GAG GAT CCA 1557

 S G P G I T G N K K N P T S K P G K N S 539
 TCA GGG CCA GGC ATC ACT GGT AAT AAA AAG AAC CCA ACC TCT AAA CCG GGG AAG AAC AGT 1617

 A S E E D H L P L Q V L Q S P * 555
 GCC TCA GAG GAA GAC CAT CTG CCC CTT CAG GTC CTC CAG TCC CCC TGA 1665

 TGGCCAGATGACAGCAGGCTGCGAGGATGGAGTAGGGAATCTTCCGAGCCACACAGAGGCTACTGAATTTTGGTG
 GAAATATTAATTTTTTTTGCATATAAAAAAAGGGGGGCGC

FIGURE 4 (continued)

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GAP of: humanvr2.pep check: 5746 from: 1 to: 764

humanVR2 Flh21e11

to: humanvr1.pep check: 6877 from: 1 to: 839

humanVR1 _Fbh18547pat - fchrb87a6, 3909 bases, 4554 checksum.

Symbol comparison table:
 /ddm_local/gcg/gcg_9.1/gcgcore/data/rundata/blosum62.cmp
 CompCheck: 6430

Gap Weight:	12	Average Match:	2.912
Length Weight:	4	Average Mismatch:	-2.003
Quality:	1530	Length:	850
Ratio:	2.003	Gaps:	10
Percent Similarity:	55.378	Percent Identity:	46.348

Match display thresholds for the alignment(s):

| = IDENTITY
 : = 2
 . = 1

humanvr2.pep x humanvr1.pep

```

1 .....MTSPSSSPVF 10
1 MKKWSSTDLGTAADPLQKDTCPDPLDGDPNRPPPAKPQLPTAKSRTLRF 50
11 RLETLDGGQEDGSEADRGKLDGSGLPPEMQFQGEDRKFAPOIRVNLNY 60
51 GKGDSEEAFFVDCPHEEGELDSCPTI.TVSPVITIQRPGDGPTGARLLSQ 99
61 RKG TGASQDPNRFDRDLFNAVSRGVPEDLAGLPEYLSKTSKYLTDSY 110
100 DSVAASTEKTLRLYDRRSIFEAVAQNNCQDLESLLLFLQSKKHLTDNEF 149
111 TEGSTGKTCLMKAVLNLDGVNACILPLLQIDRDSGNPQPLVNAQCTDDY 160
150 KDPETGKTCLLKAMNLHDGQNTTIPLLLEIARQTDSELKELVNASYTDSY 199
161 YRGHSALHIAIEKRSIQCVKLLVENGANVHARACGRFFQKGQG.TCFYFG 209
200 YKGQTALHIAIERRNMALVTLLVENGADVQAAAHGDFFKTKGRPGFYFG 249
210 ELPLSLAACTKQWDVVSYLENPHQPASLQATDSQGNTVLHALVMISDNS 259
250 ELPLSLAACTNQLGIVKFLQNSWQTADISARDSVGNTVLHALVEVADNT 299
260 AENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKLAKEGKIEI 309
300 ADNTKFVTSMYNEILMLGAKLHPTLKLLELTNKKGMTPLALAAGTGKIGV 349

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FIGURE 5

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310 FRHILQREFS..GLSHLSRKFTWCYGPVRVSLYDLASVDSCEENSVLEI 357
   :|||||      ||||||||| |||  ||||. :|.||.||||:
350 LAYILQREIQEPECRHLRSRKFTWAYGPHSSLYDLSCIDTCEKNSVLEV 399

358 IAF.HCKSPHRHRMVVLEPLNKLLOAKWDLIPK.FFLNFLCNLIYMFIF 405
   ||:  ..|.|| |...|||:|||| |||  :  : |: |||  :|| ||
400 IAYSSSETPNRHDMLLVEPLNRLLQDKWDRFVKRIFYFNFLVYCLYMIIF 449

406 TAVAYHQPTLKKQAAPHLKAEVGNMMLLTGHILILLGGIYLLVGQLWYFW 455
   |  ||:.|  .|  .:|.  .|| ||  .|||:|  :  ||
450 TMAAYYRPV..DGLPPFKMEKIGDYFRVTGEILSVLGGVYFFFRGIQYFL 497

456 RRHVFIWISFIDSYFEILFLFQALLTVVSQVLCFLAIEWYLPLLVSALVL 505
   .|  .  |:|||| |.|| |.|  .  || |  :. |.  :|. |. |
498 QRRPSMKTFLVDSYSEMLFFLQSLFMLATVVLYFSHLKEYVASMVFSAL 547

506 GWLNLLYYTRGFQHTGIYSVMIQKVILRDLLRFLLIYLVFLFGFAVALVS 555
   || |:||||||| |||.|||:||||||| ||: |:|||||. |.|.
548 GWTNMLYYTRGFQQMGIIYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVT 597

556 LSQEAWRPEAPTGPNATESVQPMEGQEDENGGAQYRGILEASLELFKFTI 605
   | ::  |.  .  |  |  .  |  :  |||||||
598 LIEDGKNDSLPSSESTSHRWGPACRPPD....SSYNSLYSTCLELFKFTI 643

606 GMGELAFQEQQLHFRGMVLLLLLAYVLLTYILLNMLIALMSETVNSVATD 655
   |||:| | | | :  .  ::|||||:||||||||| |||| :| :
644 GMGDLEFTENYDFKAVFIILLLAYVILTYILLNMLIALMGETVNKIAQE 693

656 SWSIWKLQKAISVLEMENGYWWC.RKKQKQAGVMLTVGTPDGSPDERWCF 704
   | .|||||:||.::|: |  :  | || |.| :| || ||| | ||||
694 SKNIWKLQRAITILDTEKSFLKCMRKAFRSGKLLQVGYTPDGKDDYRWCF 743

705 RVEEVNWNASWEQTLPTLCEDPSGA.GVPRTLENPVLASPPKEDEDGASEE 753
   ||:|||| |.  .  : |||  || |||  .|.  |  .
744 RVDEVNWTWNTNNGIINEDPGNCEGVKRTLSFSLRSS....RVSGRHWK 789

754 NYVPVQLLQSN..... 764
   |: | ||.
790 NFALVPLLREASARDRQSAQPEEVYLRQFSGSLKPEDAIEVFKSPAASGEK 839

```

FIGURE 5 (cont'd)

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GAP of: humanvr2.seq check: 8853 from: 1 to: 2809
 humanVR2 21e11a, 2809 bases, 8853 checksum.

to: humanvr1.seq check: 4554 from: 1 to: 3909
 humanVR1 Fbh18547pat - Import - complete

Symbol comparison table:
 /ddm_local/gcg/gcg_9.1/gcgcore/data/rundata/nwsgapdna.cmp
 CompCheck: 8760

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	0.000
Quality:	14359	Length:	3934
Ratio:	5.112	Gaps:	15
Percent Similarity:	55.316	Percent Identity:	55.316

Match display thresholds for the alignment(s):

| = IDENTITY
 : = 5
 . = 1

humanvr2.seq x humanvr1.seq

```

      .
      .
      .
1 .....GGCTAGCCTGTCCTGACAGGGGAGAG 26
      | | | | | | | |
801 TGTCCACAGTAGTCCCCCCTTATCCACGGGTGTCACTTTCCATGGGTTCA 850
      .
      .
27 TTAAGCTCCCGTTCTCCACCGTGCCGGCTGGCCAGGTGGGCTGAGGGTGA 76
      | | | | | | | | | | | |
851 GTTATTTGCGGTCAACCACGGTCTGCCAATATTAAATGGAAAATTCTTCA 900
      .
      .
77 CCGAGAGACCAGAACCTGCTTGCTGGAGCTTAGTGCTCAGAGCTGGGGAG 126
      | | | | | | | | | | | |
901 AACAGTTCCCAAGTTTTCCCTTGTGCATTGTTCTGAGCAGTGTGATGAAG 950
      .
      .
127 GGAGGTTCCGCCGCTCCTCTGCTGTCAGCGCCGGCAGCCCCTCCCGGCTT 176
      | | | | | | | | | | | |
951 AGTCTCTGCCGTGCCATCTGGGATGCAAACCGTCCCTGTGTCCCCCACGT 1000
      .
      .
177 CACTTCCTCCCGCAGCCCCTGCTACTGAGAAGCTCCGGGATCCCAGCAGC 226
      | | | | | | | | | | | |
1001 CCAGGCCGTAGATGCTCCCGCCGGTCAGTCACTTAGTCGTCAGATCGCC 1050
      .
      .
227 CGCCACGCCCTGGC.....CTCAGCCTGCGGG 253
      | | | | | | | | | | | |
1051 CGTCCTGGTATCACAGTGCTTCTGTTTCAGGTTGCACACTGGGCCACAGAG 1100
      .
      .
      .

```

FIGURE 6

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254 GCTCCAGTCAGGCCAACACCGACGCGCAGCTGGGAGGAAG..... 293
| | | | | | | | | | | | | | | | | | | | | |
1101 GATCCAGCAAGGATGAAGAAATGGAGCAGCACAGACTTGGGGACAGCTGC 1150

294ACAGGACCCTTGACATCTCCATCTGCACAGAGGTCCTG 331
| | | | | | | | | | | | | | | | | | | | | |
1151 GGACCCACTCCAAAAGGACACCTGCCCAGACCCCTGGATGGAGACCCTA 1200

332 GCTGGACCGAGCAGCCTCCTCCTAGGATGACCTCACCTCCAGC..T 379
| | | | | | | | | | | | | | | | | | | | | |
1201 ACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCCCCACGGCCAAGAGCCGC 1250

380 CTCCAGTTTTCAGGTTGGAGACATTAGATGGAGGCCAAGAAGATGGCTCT 429
| | | | | | | | | | | | | | | | | | | | | |
1251 ACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTCCCGGTGGATTG 1300

430 GAGGCGGACAGAGGAAAGCTGGATTTTGGGAGCGGGCTGCCTCCCATGGA 479
| | | | | | | | | | | | | | | | | | | | | |
1301 CCCCCACGAGGAAGGTGAGTTGGACTCCTGCCCGACCATCACAGTCAGCC 1350

480 GTCACAGTTCCAGGGCGAGGACCGGAAATTCGCCCCCTCAGATAAGAGTCA 529
| | | | | | | | | | | | | | | | | | | | | |
1351 CTGTTATCACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGG..C 1398

530 ACCTCAACTACCGAAAGGGAACAGGTGCCAGTCAGCCGGATCCAAACCGA 579
| | | | | | | | | | | | | | | | | | | | | |
1399 TGCTGTCCCAGGACTCTGTGCGCGCCAGCACCGAGAAGACCCTCAGGCTC 1448

580 TTTGACCGAGATCGGCTCTTCAATGCGGTCTCCCGGGGTGTCCCCGAGGA 629
| | | | | | | | | | | | | | | | | | | | | |
1449 TATGATCGCAGGAGTATCTTTGAAGCCGTTGCTCAGAATAACTGCCAGGA 1498

630 TCTGGCTGGACTTCCAGAGTACCTGAGCAAGACCAGCAAGTACCTCACCG 679
| | | | | | | | | | | | | | | | | | | | | |
1499 TCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAGCACCTCACAG 1548

680 ACTCGGAATACACAGAGGGCTCCACAGGTAAGACGTGCCTGATGAAGGCT 729
| | | | | | | | | | | | | | | | | | | | | |
1549 ACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC 1598

730 GTGCTGAACCTTAAGGACGGAGTCAATGCCTGCATTCTGCCACTGCTGCA 779
| | | | | | | | | | | | | | | | | | | | | |
1599 ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGA 1648

780 GATCGACAGGGACTCTGGCAATCCTCAGCCCCCTGGTAAATGCCAGTGCA 829
| | | | | | | | | | | | | | | | | | | | | |
1649 GATCGCGCGGCAAACGGACAGCCTGAAGGAGCTTGTCAACGCCAGCTACA 1698

830 CAGATGACTATTACCGAGGCCACAGCGCTCTGCACATCGCCATTGAGAAG 879
| | | | | | | | | | | | | | | | | | | | | |
1699 CGGACAGCTACTACAAGGGCCAGACAGCACTGCACATCGCCATCGAGAGA 1748

880 AGGAGTCTGCAGTGTGTGAAGCTCCTGGTGGAGAATGGGGCCAATGTGCA 929
| | | | | | | | | | | | | | | | | | | | | |
1749 CGCAACATGGCCCTGGTGACCTCCTGGTGGAGAACGGAGCAGACGTCCA 1798

930 TGCCCGGGCCTGCGGCCGCTTCTTCCAGAAGGGCCAAG...GGACTTGCT 976
| | | | | | | | | | | | | | | | | | | | | |
1799 GGCTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGGCGGCCTGGAT 1848

FIGURE 6 (cont'd)

977 TTTATTTTCGGGTGAGCTACCCCTCTCTTTGGCGCTTGCAACCAAGCAGTGG 1026
| | | | | | | | | | | | | | | | | | | | | |
1849 TCTACTTCGGGTGAAGTGCCTCTGCCCTGGCCGCGTGCACCAACCAGCTG 1898

1027 GATGTGGTAAGCTACCTCCTGGAGAACCCACACCAGCCC GCCAGCCTGCA 1076
| | | | | | | | | | | | | | | | | | | | | |
1899 GGCATCGTGAAGTTCTCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAG 1948

1077 GGCCA CTGACTCCCAGGGCAACACAGTCTTGCATGCCCTAGTGATGATCT 1126
| | | | | | | | | | | | | | | | | | | | | |
1949 CGCCAGGGACTCGGTGGGCAACACGGTGTCTGCACGCCCTGGTGGAGGTGG 1998

1127 CGGACA ACTCAGCTGAGA ACATTGCACTGGTGACCAGCATGTATGATGGG 1176
| | | | | | | | | | | | | | | | | | | | | |
1999 CCGACAACACGGCCGACAACACGAAGTTTGTGACGAGCATGTACAATGAG 2048

1177 CTCCTCCAAGCTGGGGCCCCGCTCTGCCCTACCGTGCAGCTTGAGGACAT 1226
| | | | | | | | | | | | | | | | | | | | | |
2049 ATTCTGATGCTGGGGGGCAAAC TGACCCGACGCTGAAGCTGGAGGAGCT 2098

1227 CCGCAACCTGCAGGATCTCACGCCTCTGAAGCTGGCCGCCAAGGAGGGCA 1276
| | | | | | | | | | | | | | | | | | | | | |
2099 CACCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCTGGGACCGGGA 2148

1277 AGATCGAGATTTTCAGGCACATCCTGCAGCGGGAGTT..... TTCAGGA 1320
| | | | | | | | | | | | | | | | | | | | | |
2149 AGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAG 2198

1321 CTGAGCCACCTTTCCCGAAA GTTCACCGAGTGGTGCTATGGGCCTGTCCG 1370
| | | | | | | | | | | | | | | | | | | | | |
2199 TGCAGGCACCTGTCCAGGAAGTTACCGAGTGGGCCTACGGGCCCGTGCA 2248

1371 GGTGTCGCTGTATGACCTGGCTTCTGTGGACAGCTGTGAGGAGAACTCAG 1420
| | | | | | | | | | | | | | | | | | | | | |
2249 CTCCTCGCTGTACGACCTGTCCTGCATCGACACCTGCGAGAAGAACTCGG 2298

1421 TGCTGGAGATCATTGCCTTTCATTGCA... AGAGCCCGCACCCGACACCGA 1467
| | | | | | | | | | | | | | | | | | | | | |
2299 TGCTGGAGGTGATCGCCTACAGCAGCAGCGAGACCCCTAATCGCCACGAC 2348

1468 ATGGTTCGTTTTGGAGCCCCCTGAACAAACTGCTGCAGGCGAAATGGGA... 1514
| | | | | | | | | | | | | | | | | | | | | |
2349 ATGCTCTTGGTGGAGCCGCTGAACCGACTCCTGCAGGACAAGTGGGACAG 2398

1515 TCTGCTCATCCCCAAGTTCTTCTTAAACTTCCTGTGTAATCTGATCTACA 1564
| | | | | | | | | | | | | | | | | | | | | |
2399 ATTCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTACTGCCTGTACA 2448

1565 TGTT CATCTTCAACCGCTGTTGCCTACCATCAGCCTACCCCTGAAGAAGCAG 1614
| | | | | | | | | | | | | | | | | | | | | |
2449 TGATCATCTTCAACCATGGCTGCCTACTA.... CAGGCCCGTGGATGGCTT 2494

1615 GCCGCCCCCTCACCTGAAAGCGGAGGTTGGAAACTCCATGCTGCTGACGGG 1664
| | | | | | | | | | | | | | | | | | | | | |
2495 GCCTCCCTTTA..AGATGGAAAAAATTGGAGACTATTTCCGAGTTACTGG 2542

BNSDOCID: <WO _0029577A1.1 >

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1665 CCACATCCTTATCCTGCTAGGGGGGATCTACCTCCTCGTGGGCCAGCTGT 1714
| | | | | | | | | | | | | | | | | |
2543 AGAGATCCTGTCTGTGTTAGGAGGAGTCTACTTCTTTTCCGAGGGATTCT 2592
1715 GGTACTTCTGGCGGCGCCACGTGTTTCATCTGGATCTCGTTCATAGACAGC 1764
| | | | | | | | | | | | | | | | | |
2593 AGTATTTCTGTCAGAGGCGGCCGTCGATGAAGACCCTGTTTGTGGACAGC 2642
1765 TACTTTGAAATCCTCTTCTGTTCCAGGCCCTGCTCACAGTGGTGTCCCA 1814
| | | | | | | | | | | | | | | | | |
2643 TACAGTGAGATGCTTTTCTTTCTGTCAGTCACTGTTTCATGCTGGCCACCGT 2692
1815 GGTGCTGTGTTTCTGGCCATCGAGTGGTACCTGCCCTGCTTGTGTCTG 1864
| | | | | | | | | | | | | | | | | |
2693 GGTGCTGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTATTCT 2742
1865 CGCTGGTGTCTGGGCTGGCTGAACCTGCTTTACTATACACGTGGCTTCCAG 1914
| | | | | | | | | | | | | | | | | |
2743 CCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAG 2792
1915 CACACAGGCATCTACAGTGTCATGATCCAGAAGGTCATCCTGCGGGACCT 1964
| | | | | | | | | | | | | | | | | |
2793 CAGATGGGCATCTATGCCGTGTCATGATAGAGAAGATGATCCTGAGAGACCT 2842
1965 GCTGCGCTTCCTTCTGATCTACTTAGTCTTCCTTTTCGGCTTCGCTGTAG 2014
| | | | | | | | | | | | | | | | | |
2843 GTGCCGTTTCATGTTTGTCTACATCGTCTTCTTGTTCGGGTTTTCACAG 2892
2015 CCCTGGTGAGCCTGAGCCAGGAGGCTTGGCGCCCCGAAGCTCCTACAGGC 2064
| | | | | | | | | | | | | | | | | |
2893 CGGTGGTGACGCTGATTGAAGACGGGAAGAATGACTCCCTGCCGTCTGAG 2942
2065 CCCAATGCCACAGAGTCAGTGCAGCCCATGGAGGGACAGGAGGACGAGGG 2114
| | | | | | | | | | | | | | | | | |
2943 TCCA.....CGTCGCACAGGTGGCGGGGGCCTGCCTGCAGGCC 2980
2115 CAACGGGGGCCAGTACAGGGGTATCCTGGAAGCCTCCTTGGAGCTCTTCA 2164
| | | | | | | | | | | | | | | | | |
2981 CCCCAGATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGTTCA 3030
2165 AATTCACCATCGGCATGGGCGAGCTGGCCTTCCAGGAGCAGCTGCACTTC 2214
| | | | | | | | | | | | | | | | | |
3031 AGTTCACCATCGGCATGGGCGACCTGGAGTTCACTGAGAACTATGACTTC 3080
2215 CGCGGCATGGTGTCTGCTGCTGCTGCTGGCCTACGTGCTGCTCACCTACAT 2264
| | | | | | | | | | | | | | | | | |
3081 AAGGCTGTCTTCATCATCCTGCTGCTGGCCTATGTAATTCTCACCTACAT 3130
2265 CCTGCTGCTCAACATGCTCATCGCCCTCATGAGCGAGACCGTCAACAGTG 2314
| | | | | | | | | | | | | | | | | |
3131 CCTCCTGCTCAACATGCTCATCGCCCTCATGGGTGAGACTGTCAACAAGA 3180
2315 TCGCCACTGACAGCTGGAGCATCTGGAAGCTGCAGAAAGCCATCTCTGTC 2364
| | | | | | | | | | | | | | | | | |
3181 TCGCACAGGAGAGCAAGAACATCTGGAAGCTGCAGAGAGCCATCACCATC 3230

FIGURE 6 (cont'd)

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2365 CTGGAGATGGAGAATGGCTATTGGTGGTGCAGGAAGAAG...CAGCGGGC 2411
| | | | | | | | | | | | | | | | | | | | | |
3231 CTGGACACGGAGAAGAGCTTCCTTAAGTGCATGAGGAAGGCCTTCCGCTC 3280
2412 AGGTGTGATGCTGACCGTTGGCACTAAGCCAGATGGCAGCCCGGATGAGC 2461
| | | | | | | | | | | | | | | | | | | | | |
3281 AGGCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACTACC 3330
2462 GCTGGTGCCTTCAGGGTGGAGGAGGTGAACTGGGCTTCATGGGAGCAGACG 2511
| | | | | | | | | | | | | | | | | | | | | |
3331 GGTGGTGCCTTCAGGGTGGACGAGGTGAACTGGACCACCTGGAACACCAAC 3380
2512 CTGCCTACGCTGTGTGAGGACCCG...TCAGGGGCAGGTGTCCCTCGAAC 2558
| | | | | | | | | | | | | | | | | | | | | |
3381 GTGGGCATCATCAACGAAGACCCGGGCAACTGTGAGGGCGTCAAGCGCAC 3430
2559 TCTCGAGAACCCTGTCCTG...GCTTCCCCTCCCAAGGAGGATGAGGAT 2604
| | | | | | | | | | | | | | | | | | | | | |
3431 CCTGAGCTTCTCCCTGCGGTCAAGCAGAGTTTCAGGCAGACACTGGAAGA 3480
2605 GGTGCCTCTGAGGAAAACCTATGTGCCCGTCCAGCTCCTCCAGTCCAACCTG 2654
| | | | | | | | | | | | | | | | | | | | | |
3481 ACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCTCGAGATAGGCAG 3530
2655 ATGGCCCAGATGCAGCAGGAGGCCAGAGGACAGAGCAGAGGATCTTTCCA 2704
| | | | | | | | | | | | | | | | | | | | | |
3531 TCTGCTCAGCCCCGAGGAAGTTTATCTGCGACAGTTTTCAGGGTCTCTGAA 3580
2705 ACCACATCTGCTGGCTCTGGGGTCCAGTGAATTCTGGTGGCAAATATAT 2754
| | | | | | | | | | | | | | | | | | | | | |
3581 GCCA.....GAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGGGGA 3625
2755 ATTTTCACTAACTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2804
| | | | | | | | | | | | | | | | | | | | | |
3626 GAAGTGAGGACGTCACGCAGACAGCACTGTCAACACTGGGCCTTAGGAGA 3675
2805 AAAAA..... 2809
3676 CCCCCTTGCCACGGGGGGCTGCTGAGGGAACACCAGTGCTCTGTCTCAGCAG 3725
.
.

FIGURE 6 (cont'd)

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CLUSTAL W (1.74) multiple sequence alignment

```

humanVR2      MTSPSSSPVFRLETLDGGQEDGSEADRGKLDFGSGLPPMESQFQGEDRKFAPOIRVNLNY
ratVR2      -----

humanVR2      RKG TGASQDPNRFDRDLFNAVSRGVPEDLAGLPEYLSKTSKYLTDSEYTEGSTGKTCL
ratVR2      -----

humanVR2      MKAVLNLDGVDNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLQCVK
ratVR2      -----

humanVR2      LLVENGANVHARACGRFFQKGQGTCTFYFGELPLSLAACTKQWDVVSYLLENPHQPASLQA
ratVR2      -----STHASALSLAACTKQWDVVTYLLENPHQPASLEA
                      *****:*****:

humanVR2      TDSQGNTVLHALVMISDNSAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKL
ratVR2      TDSLGNLTVLHALVMIADNSPENSALVIHMYDGLLQMGARLCPTVQLEEISNHQGLTPLKL
                      *** *****:***.*** ***** *****:***.*****

humanVR2      AAKEGKIEIFRHILQREFSG-LSHLSRKFTWCYGPVRVSLYDLASVDSCEENSVL EIIA
ratVR2      AAKEGKIEIFRHILQREFSGPYQPLSRKFTEWCYGPVRVSLYDLSSVDSWEKNSVL EIIA
                      *****:*****:*****:*****:*****:

humanVR2      FHCKSPHRHRMVVLEPLNKLLOAKWDLIPKFFLNFLCNLIYMFIFTAVAYHQPTLKKQA
ratVR2      FHCKSPNRHRMVVLEPLNKLLOEKWDLVSRFFNFACYLVYMFIFTVVAYHQPSLDQPA
                      *****:*****:*** *:***:*** *:*****:*****:*.:.

humanVR2      APHLKAEVGNMMLLTGHILILLGGIYLLVGLWYFWRRHVFIIWISFIDSIFYEILFLFOAL
ratVR2      IPSSKATFGESMMLLGHILILLGGIYLLVGLWYFWRRRLFIWISFMDSYFEILFLLOAL
                      * ** .*:*** *****:*****:*****:*****:*****:

humanVR2      LTVVSQVLCFLAIEWYLPLLVSALVLGWLNLNLLYYTRGFQHTGIYSVMIQVILRDLLRFL
ratVR2      LTVLSQVLRFMETEWYLPLLVLSVLGWLNLNLLYYTRGFQHTGIYSVMIQVILRDLLRFL
                      ***:*** *: ***** :*****:*****:*****:*****:

humanVR2      LIYLVFLFGFAVALVSLSQEAWRPEAPTGPNTATESVQPMEGQEDEGNGAQYRGILEASLE
ratVR2      LVYLVFLFGFAVALVSLSRARSPPKAPEDNNSVTQEPTVGQREKP--APYRSILDASLE
                      *:*****:*** *:*** .*: * : ** *:***:*** **.:***

humanVR2      LFKFTIGMGELAFQEQHFRGMVLLLLLAYVLLTYILLNMLIALMSETVNSVATDSWSI
ratVR2      LFKFTIGMGELAFQEQHFRGMVLLLLLAYVLLTYVLLNMLIALMSETVNHVADNSWSI
                      *****:***:*****:*****:*****:*****:***:****

humanVR2      WKIQKALSILEMENGYYWCR-KKORAGVMLTVGTPDGSPDERWCPRVEEVNWAWEQTL
ratVR2      WKIQKALSILEMENGYYWCRKKHREGRLLVGTRGDGTPDERWCPRVEEVNWAWEKTL
                      *****:***:*** *:***:***:*****:*****:***:***

humanVR2      PTLCEDPGAGVPRTLNPFVLASPPKEDDGASEENYVPVQLLOSN
ratVR2      PTLSEDPGPGITGNKNPTSK-PGR---NSASEKHLPLOVLQSP
                      ***.*****:*. .:***. ** :.*****:***:***

```

FIGURE 7

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GAP of: ratvr2.pep check: 9190 from: 1 to: 554

ratVR2 Flrxb147g11

to: humanvr2.pep check: 5746 from: 1 to: 764

humanVR2 Flh21e11

Symbol comparison table: /usr/local/gcg_9.1/gcgcore/data/rundata/blosum62.cmp
CompCheck: 6430

Gap Weight:	12	Average Match:	2.912
Length Weight:	4	Average Mismatch:	-2.003
Quality:	2182	Length:	766
Ratio:	3.939	Gaps:	4
Percent Similarity:	81.703	Percent Identity:	79.167

Match display thresholds for the alignment(s):

```

| = IDENTITY
: = 2
. = 1

```

ratvr2.pep x humanvr2.pep

```

1 .....STHASALSIACTKQWDVVITYLLENPHQPASLEATDSLGNTVLH 44
|||||
201 GQGTCTFYFPGELPLSLAACKQWDVVSYLENPHQPASLOATDSQGNVLH 250
45 ALVMIADNSPENSALVIHMYDGLLOMGARLCPTVQLEEISNHQGLTPLKL 94
|||||
251 ALVMISDNSAENIALVTSMYDGLLOAGARLCPTVQLEDIRNLQDLTPLKL 300
95 AAKEGKIEIFRHILQREFSGPYQPLSRKFTWCYGPVRVSLYDLSSVDSW 144
|||||
301 AAKEGKIEIFRHILQREFSG.LSHLSRKFTWCYGPVRVSLYDLASVDS 349
145 EKNSVLEIIAFHCKSPNRHRMVVLEPLNKLLQEKWDRLVSRFFNFACYL 194
|.|||||
350 EKNSVLEIIAFHCKSPHRHRMVVLEPLNKLLQAKWDLIPKFFLNFLCNL 399
195 VYMFIFTVVAYHQPSSLDQPAIPSSKATFGESMLLLGHILILGGIYLLG 244
:|||||
400 IYMFIFTAVAYHQPSTLKKQAAPHLKAEVGNISMLLTGHILILGGIYLLV 449
245 QLWYFWRRLFIWISFMDSYFEILFLQALLTVLSQVLRFMETEWYLPIL 294
|||||
450 QLWYFWRRHVFIWISFIDSYFEILFLQALLTVVSQVLCFLAIEWYLPIL 499
295 VLSVLGWLNLLYYTRGFQHTGIYEVMIQKVILRDLLRFLLVYLVFLGF 344
|.|||||
500 VEALVLGWLNLLYYTRGFQHTGIYEVMIQKVILRDLLRFLLVYLVFLGF 549
345 AVALVLSREARSPKAPEDNSTVTQPTVQOKEE..PAPYRSILDASLE 392
|||||
550 AVALVLSQEARPEAPTGPNTATESVQPMEGQEDGNGAQYRGILEASLE 599

```

FIGURE 8

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```

393 LFKFTIGMGELAFQEQLRFRGVVLLLLLAYVLLTYVLLLNMLIALMSETV 442
      |||||
600 LFKFTIGMGELAFQEQLFHFRGMVLLLLLAYVLLTYILLNMLIALMSETV 649
      |||||
443 NHVADNSWSIWKLQKAISVLEMENGYWWCRRKKHREGRLKVGTRGDGTP 492
      ||| .|||
650 NSVATDSWSIWKLQKAISVLEMENGYWWC.RKKORAGVMLTVGTPKPDGSP 698
      ||| .|||
493 DERWCFRVEEVNWAWEKTLPTLSEDPSGPGITGNKKNP...SKPGKN 538
      |||||
699 DERWCFRVEEVNWAWEQTLPTLCEDPGAGVPRTLENPVLASPPKEDED 748
      |||||
539 SASEEDHLPLOVLQSP 554
      |||||.:.|.|.|||
749 GASEENYVPVQLQSN 764

```

FIGURE 8 (continued)

GAP of: humanvrl.seq check: 4554 from: 1 to: 3909

to: ratvrl.seq check: 7921 from: 1 to: 2847

Symbol comparison table:

CompCheck: 8760

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	0.000
Quality:	22717	Length:	3914
Ratio:	7.979	Gaps:	10
Percent Similarity:	82.125	Percent Identity:	82.125

```
Match display thresholds for the alignment(s):
      | = IDENTITY
      : = 5
      - = 1
```

humanvrl.seq x ratvrl.seq .

1001 CCAGGCCGTAGATGCTCCCCGCCGGTCAGTCACTTAGTCGT CAGATCGCC 1050
1CAGCTCCAAGGCACTTGCTCC 21
1051 CGTCCTGGTATCACAGTGCTTCTGTT CAGGTTGCACACTGGGCCACAGAG 1100
22 ATTTGGGGTGTGCCTGCACCT...AGCTGGTTGCAAATTGGGCCACAGAG 68
1101 GATCCAGCAAGGATGAAGAAATGGAGCAGCACAGACTTGGGGACAGCTGC 1150
69 GATCTGGAAGGATGGAACAACGGGCTAGCTTAGACTCAGAGGAGTCTGA 118
1151 GGACCCACTCCAAAAGGACACCTGCC CAGACCCCCTGGATGGAGACCCTA 1200
119 GTCCCCACCCCAAGAGAACTCCTGCCTGGACCCTCCAGACAGAGACCCTA 168
1201 ACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCCCCACGGCCAAGAGCCGC 1250
169 ACTGCAAGCCACCTCCAGTCAAGCCCCACATCTTCACTACCAGGAGTCGT 218
1251 ACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGATTG 1300
219 ACCCGGCTTTTGGGAAGGGTGACTCGGAGGAGGCCTCTCCCCTGGACTG 268
1301 CCCCCACGAGGAAGGTGAGTTGGACTCCTGCCCGACCATCACAGTCAGCC 1350
269 CCCTTATGAGGAAGGCGGGCTGGCTTCCTGCCCTATCATCACTGTCAGCT 318
1351 CTGTTATCACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTG 1400
319 CTGTTCTAACTATCCAGAGGCCTGGGGATGGACCTGCCAGTGT CAGGCCG 368

FIGURE 9

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1401 CTGTCCCAGGACTCTGTGCGCCGCCAGCACCGAGAAGACCCCTCAGGCTCTA 1450
||||| ||| ||| | ||||| ||| |||||
369 TCATCCCAGGACTCCGTCTCCGCTGG...TGAGAAGCCCCCGAGGCTCTA 415
1451 TGATCGCAGGAGTATCTTTGAAGCCGTTGCTCAGAATAACTGCCAGGATC 1500
||||| ||| ||| ||| ||| ||||| ||||| |||||
416 TGATCGCAGGAGCATCTTCGATGCTGTGGCTCAGAGTAACTGCCAGGAGC 465
1501 TGGAGAGCCTGCTGCTCTTCTCCTGCAGAAGAGCAAGAAGCACCTCACAGAC 1550
||||| ||||| ||||| ||||| ||||| ||||| |||||
466 TGGAGAGCCTGCTGCCCTTCTCCTGCAGAGGAGCAAGAAGCGCCTGACTGAC 515
1551 AACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCCAT 1600
| ||||| ||||| ||||| ||||| ||||| ||||| |||||
516 AGCGAGTTCAAAGACCCAGAGACAGGAAAGACCTGTCTGCTAAAAGCCAT 565
1601 GCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGA 1650
||||| ||||| | || ||||| ||||| | ||||| |||||
566 GCTCAATCTGCACAATGGGCAGAATGACACCATCGCTCTGCTCCTGGACG 615
1651 TCGCGCGGCAAACGGACAGCCTGAAGGAGCTTGTCAACGCCAGCTACACG 1700
| || ||| | || ||||| ||||| || ||||| ||||| |||||
616 TTGCCCCGAAGACAGACAGCCTGAAGCAGTTTGTCAATGCCAGCTACACA 665
1701 GACAGCTACTACAAGGGCCAGACAGCACTGCACATCGCCATCGAGAGACG 1750
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
666 GACAGCTACTACAAGGGCCAGACAGCACTGCACATTGCCATTGAACGGCG 715
1751 CAACATGGCCCTGGTGACCCTCCTGGTGGAGAACGGAGCAGACGTCCAGG 1800
||||| | ||||| ||||| ||||| ||||| ||||| ||||| |||||
716 GAACATGACGCTGGTGACCCTCTTGGTGGAGAATGGAGCAGATGTCCAGG 765
1801 CTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGGCGGCCTGGATTTC 1850
||||| | ||||| ||||| ||||| ||||| ||||| ||||| |||||
766 CTGCGGCTAACGGGGACTTCTTCAAGAAAACCAAAGGGAGGCCTGGCTTC 815
1851 TACTTCGGTGAAGTGCCCCCTGTCCCTGGCCGCGTGCACCAACCAGCTGGG 1900
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
816 TACTTTGGTGAGCTGCCCCCTGTCCCTGGCTGCGTGCACCAACCAGCTGGC 865
1901 CATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCG 1950
||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
866 CATTGTGAAGTTCCTGCTGCAGAACTCCTGGCAGCCTGCAGACATCAGCG 915
1951 CCAGGGACTCGGTGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGGCC 2000
|| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
916 CCCGGGACTCAGTGGGCAACACGGTGCTTCATGCCCTGGTGGAGGTGGCA 965
2001 GACAACACGGCCGACAACACGAAGTTTGTGACGAGCATGTACAATGAGAT 2050
|| ||||| | ||||| ||||| ||||| ||||| ||||| ||||| |||||
966 GATAACACAGTTGACAACACCAAGTTTCGTGACAAGCATGTACAACGAGAT 1015
2051 TCTGATGCTGGGGGCCAAACTGCACCCGACGCTGAAGCTGGAGGAGCTCA 2100
|||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1016 CTTGATCCTGGGGGCCAAACTCCACCCACGCTGAAGCTGGAAGAGATCA 1065

FIGURE 9 (cont'd)

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2101 CCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCTGGGACCGGGAAG 2150
||||| ||||| | ||||| ||||| ||||| ||||| ||||| |||||

1066 CCAACAGGAAGGGGCTCACGCCACTGGCTCTGGCTGCTAGCAGTGGGAAG 1115

2151 ATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAGTG 2200
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

1116 ATCGGGGTCTTGGCCTACATTCTCCAGAGGGAGATCCATGAACCCGAGTG 1165

2201 CAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCCGTGCACT 2250
| | ||||| ||||| ||||| ||||| ||||| ||||| |||||

1166 CCGACACCTATCCAGGAAGTTCACCGAATGGGCCTATGGGCCAGTGCACCT 1215

2251 CCTCGCTGTACGACCTGTCCTGCATCGACACCTGCGAGAAGAACTCGGTG 2300
|||| | | ||||| ||||| ||||| ||||| ||||| |||||

1216 CCTCCCTTTATGACCTGTCCTGCATTGACACCTGTGAAAAGAACTCGGTT 1265

2301 CTGGAGGTGATCGCCTACAGCAGCAGCGAGACCCCTAATCGCCACGACAT 2350
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

1266 CTGGAGGTGATCGCTTACAGCAGCAGTGAGACCCCTAACCGTCATGACAT 1315

2351 GCTCTTGGTGGAGCCGCTGAACCGACTCCTGCAGGACAAGTGGGACAGAT 2400
|| | ||||| || ||||| ||||| ||||| ||||| |||||

1316 GCTTCTCGTGGAACCCCTGAACCGACTCCTACAGGACAAGTGGGACAGAT 1365

2401 TCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTACTGCCTGTACATG 2450
| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

1366 TTGTCAAGCGCATCTTCTACTTCAACTTCTTCGTCTACTGCTTGTATATG 1415

2451 ATCATCTTCACCATGGCTGCCTACTACAGGCCCGTGGATGGCTTGCCTCC 2500
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

1416 ATCATCTTCACCGCGGCTGCCTACTATCGGCCTGTGGAAGGCTTGCCCC 1465

2501 CTTTAAGATGGAAAAA...TTGGAGACTATTTCCGAGTTACTGGAGAGA 2547
|| |||| | |||| | |||| ||||| ||||| ||||| |||||

1466 CTATAAGCTGAAAAACACCGTTGGGGACTATTTCCGAGTCACCGGAGAGA 1515

2548 TCCTGTCTGTGTTAGGAGGAGTCTACTTCTTTTCCGAGGGATTCAGTAT 2597
|| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

1516 TCTTGTCTGTGTCAGGAGGAGTCTACTTCTTCTTCCGAGGGATTCAATAT 1565

2598 TTCCTGCAGAGGCGGCCGTCGATGAAGACCCTGTTTGTGGACAGCTACAG 2647
||||| ||||| || || | |||| | ||||| ||||| |||||

1566 TTCCTGCAGAGGCGACCATCCCTCAAGAGTTTGTGTTGTGGACAGCTACAG 1615

2648 TGAGATGCTTTTCTTTCTGCAGTCACTGTTTCATGCTGGCCACCGTGGTGC 2697
||||| ||||| || |||| | |||| | ||||| ||||| |||||

1616 TGAGATACTTTTCTTTGTACAGTCGCTGTTTCATGCTGGTGTCTGTGGTAC 1665

2698 TGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTATTCTCCCTG 2747
||||| ||||| || ||||| ||||| ||||| ||||| ||||| |||||

1666 TGTACTTCAGCCAACGCAAGGAGTATGTGGCTTCCATGGTGTCTCCCTG 1715

2748 GCCTTGGGCTGGACCAACATGCTCTACTACACCGCGGTTTCCAGCAGAT 2797
|| | ||||| ||||| ||||| ||||| ||||| ||||| |||||

1716 GCCATGGGCTGGACCAACATGCTCTACTATACCGAGGATTCCAGCAGAT 1765

FIGURE 9 (cont'd)

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2798 GGGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCC 2847
|||||
1766 GGGCATCTATGCTGTCATGATTGAGAAGATGATCCTCAGAGACCTGTGCC 1815
2848 GTTTCATGTTTGTCTACATCGTCTTCTTGTTTCGGGTTTTCCACAGCGGTG 2897
| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
1816 GGTTCATGTTTCGTCTACCTCGTGTCTTGTTCGATTTCACAGCTGTG 1865
2898 GTGACGCTGATTGAAGACGGGAAGAATGACTCCCTGCCGCTGAGTCCAC 2947
||||| ||||| ||||| ||||| ||||| ||||| |||||
1866 GTGACACTGATTGAGGATGGGAAGAATAACTCTCTGCCTATGGAGTCCAC 1915
2948 GTCGCACAGGTGGCGGGGGCCTGCCTGCAGGCCCCCGATAGCTCCTACA 2997
| |||| | ||||| ||||| ||||| ||||| ||||| |||||
1916 ACCACACAAGTGCCGGGGGTCTGCCTGCAAG...CCAGGTAACCTTTACA 1962
2998 ACAGCCTGTACTCCACCTGCCTGGAGCTGTTCAAGTTCACCATCGGCATG 3047
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1963 ACAGCCTGTATTCCACATGTCTGGAGCTGTTCAAGTTCACCATCGGCATG 2012
3048 GGCGACCTGGAGTTCACTGAGAAGTATGACTTCAAGGCTGTCTTCATCAT 3097
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
2013 GGCGACCTGGAGTTCACTGAGAAGTACGACTTCAAGGCTGTCTTCATCAT 2062
3098 CCTGCTGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGC 3147
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
2063 CCTGTTACTGGCCTATGTGATTCTCACCTACATCCTTCTGCTCAACATGC 2112
3148 TCATCGCCCTCATGGGTGAGACTGTCAACAAGATCGCACAGGAGAGCAAG 3197
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
2113 TCATTGCTCTCATGGGTGAGACCGTCAACAAGATTGCACAAGAGAGCAAG 2162
3198 AACATCTGGAAGCTGCAGAGAGCCATCACCATCCTGGACACGGAGAAGAG 3247
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
2163 AACATCTGGAAGCTGCAGAGAGCCATCACCATCCTGGATACAGAGAAGAG 2212
3248 CTTCTTAAGTGCATGAGGAAGGCCTTCCGCTCAGGCAAGCTGCTGCAGG 3297
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
2213 CTTCTGAAGTGCATGAGGAAGGCCTTCCGCTCTGGCAAGCTGCTGCAGG 2262
3298 TGGGGTACACACCTGATGGCAAGGACGACTACCGGTGGTGCTTCAGGGTG 3347
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
2263 TGGGGTTCACCTCCTGACGGCAAGGATGACTACCGGTGGTGTTTCAGGGTG 2312
3348 GACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCATCATCAACGA 3397
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
2313 GACGAGGTAACTGGACTACCTGGAACACCAATGTGGGTATCATCAACGA 2362
3398 AGACCCGGGCAACTGTGAGGGCGTCAAGCGCACCCCTGAGCTTCTCCCTGC 3447
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
2363 GGACCCAGGCAACTGTGAGGGCGTCAAGCGCACCCCTGAGCTTCTCCCTGA 2412
3448 GGTCAAGCAGAGTTTCAGGCAGACACTGGAAGAACTTTGCCCTGGTCCCC 3497
||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
2413 GGTCAGGCCGAGTTTCAGGGAGAACTGGAAGAACTTTGCCCTGGTCCCC 2462
3498 CTTTTAAGAGAGGCAAGTGCTCGAGATAGGCAGTCTGCTCAGCCCGAGGA 3547

FIGURE 9 (cont'd)

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```

    ||| | || || |||| | |||| || | | ||| || |
2463 CTTCTGAGGGATGCAAGCACTCGAGATAGACATGCCACCCAGCAGGAAGA 2512
    .
3548 AGTTTATCTGCGACAGTTTTTCAGGGTCTCTGAAGCCAGAGGACGCTGAGG 3597
    |||| | || | | | | | | | | | | | | | | | |
2513 AGTTCAACTGAAGCATTATACGGGATCCCTTAAGCCAGAGGATGCTGAGG 2562
    .
3598 TCTTCAAGAGTCCTGCCGCTTCCGGGGAGAAAGTGA.GGACGTCACGCAGA 3646
    | |||| | | | | | | | | | | | | | | | |
2563 TTTTCAAGGATTCCATGGTCCCAGGGGAGAAATAATGGACACTATGCAGG 2612
    .
3647 CAGCACTGTCAACACTGGGCCTTAGGAGACCCCGTTGCCACGGGGGGCTG 3696
    | || | | | | | | | | | | | | | | | |
2613 GATCAATG.....CGGGGTCTTTGGGTGGTCTG 2640
    .
3697 CTGAGGGAACACCACTGCTCTGTCTCAGCAGCCTGGCCTGGTCTGTGCCTGC 3746
    || |||| | | | | | | | | | | | | | | | |
2641 CTTAGGGAAC.CAGCAGGGTTGACGTTATCTGGGTCCACTCTGTGCCTGC 2689
    .
3747 CCA.GCATGTTCCCAAATCTGTGCTGGACAAGCTGTGGGAAGCGTTCTTG 3795
    | | || | || | | | | | | | | | | | |
2690 CTAGGCACATTCCCTAGGACTTCGGCGGGCCTGCTGTGGGAA.CTGGGAGG 2738
    .
3796 GAAGCATGGGGAGTGATGTACATCCAACCGTCACTGTCCCAAGTGAATC 3845
    | | | | | |||| | |||| | | | | | | |
2739 TGTGTGGGAATTGAGATGTGTATCCAACCATGA...TCTCCAAACATTTG 2785
    .
3846 TCCTAACAGACTTTCAGGTTTTTACTCACTTTACTAAAAAAAAAAAAAAAA 3895
    | | | | |||| | | |||| | | | | | | |
2786 GCTTTCAACTCTTTATGGACTTTATTAAACAGAGTGAATGGCAAATCTCT 2835
    .
3896 AGGGCGGCCGCTTA 3909
    | | | |
2836 ACTTGGACACAT.. 2847
```

FIGURE 9 (cont'd)

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GAP of: humanvr1.pep check: 6877 from: 1 to: 839
 humanVR1_Fbh18547pat - fchrb87a6, 3909 bases, 4554 checksum.
 to: ratvr1.pep check: 5764 from: 1 to: 838
 ratVR1 | AF029310 Rattus norvegicus vanilloid receptor subtype 1 mRNA,
 complete
 cds.

Symbol comparison table:
 /ddm_local/gcg/gcg_9.1/gcgcore/data/rundata/blosum62.cmp
 CompCheck: 6430

Gap Weight:	12	Average Match:	2.912
Length Weight:	4	Average Mismatch:	-2.003
Quality:	3734	Length:	840
Ratio:	4.456	Gaps:	3
Percent Similarity:	89.247	Percent Identity:	86.022

Match display thresholds for the alignment(s):
 | = IDENTITY
 : = 2
 . = 1

humanvr1.pep x ratvr1.pep

```

1 MKKWSSTD LGTAADPLQKDTCPDPLDGD PNSRPPPAKPQLPTAKSRTRLF 50
  |.. .| | . | |...| || | ||| :||| || : | :| |||||
1 MEQRASLDSEESPPQENSCLDPPDRDPNCKPPPVKPHIFTTRSRTRLF 50

51 GKGDSEEAFFVDCPHEEGELDSCPTITVSPVITIQRPGDGPTGARLLSQD 100
  ||||| | |. |||: ||| | ||| ||| | :||| ||||| | |||
51 GKGDSEEA SPLDCPYEEGGLASCPITVSSVLT IQRPGDGPASVRPSSQD 100

101 SVAASTEKTLRLYDRRSIFEAVAQNNCQDLESLLLFLQSKKKHLTDNEFK 150
  ||. | || | ||||| :||| . |||: |||| | |||: ||| |||. |||
101 SVSAG.EKPPRLYDRRSIFDAVAQSNQCELESLLPFLQRSKKRLTDSEFK 149

151 DPETGKTCLLKAMNLNHDGQNTTIPLLLEIARQTD SLKELVNASYTDSYY 200
  ||||| ||||| ||||| . ||| || |||: ||. ||||| : ||||| |||||
150 DPETGKTCLLKAMNLNHNQNDTIALLLDVARKTDSLKQFVNASYTDSYY 199

201 KGQTALHIAIERRNMALVTLLVENGADVQAAAAGDFFKKTGKRPGFYFGE 250
  ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
200 KGQTALHIAIERRNMTLVTLVENGADVQAAANGDFFKKTGKRPGFYFGE 249

251 LPLSLAACTNQLGIVKFLQNSWQTADISARDSVGNTVLHALVEVADNTA 300
  ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
250 LPLSLAACTNQLAIVKFLQNSWQPADISARDSVGNTVLHALVEVADNTV 299

301 DNTKFVTSMYNEILMLGAKLHPTLKLEELTNKKGMTPLALAAGTGKIGVL 350
  ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
300 DNTKFVTSMYNEILILGAKLHPTLKLEETNRKGLTPLALAASSGKIGVL 349

351 AYILQREIQEPECRHLSRKFTWAYGVPVHSSLYDLSCIDTCEKNSVLEVI 400
  ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
350 AYILQREIHEPECRHLSRKFTWAYGVPVHSSLYDLSCIDTCEKNSVLEVI 399

```

FIGURE 10

401	AYSSSETPNRHDMLLVEPLNRLQLQDKWDRFVKRIFYFNFLVYCLYMIIFT	450
400	AYSSSETPNRHDMLLVEPLNRLQLQDKWDRFVKRIFYFNFFVYCLYMIIFT	449
451	MAAYYRPVDGLPPFKMEK.IGDYFRVTGEILSVLGGVYFFFRGIQYFLQR	499
	: : : : :	
450	AAAYYRPVEGLPPYKLKNTVGDYFRVTGEILSVSGGVYFFFRGIQYFLQR	499
500	RPSMKTLFVDSYSEMLFFLQSLFMLATVVLYFSHLKEYVASMVFSLALGW	549
	:	
500	RPSLKSFLVDSYSEILFFVQSLFMLVSVVLYFSQRKEYVASMVFSLAMGW	549
550	TNMLYYTRGFQQMGIYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVTLI	599
550	TNMLYYTRGFQQMGIYAVMIEKMILRDLCRFMFVYLVFLFGFSTAVVTLI	599
600	EDGKNDSLPESTSHRWRGPACRPPDSSYNSLYSTCLELFKFTIGMGDLE	649
	. : : .	
600	EDGKNNSLPMESTPHKCRGSACK.PGNSYNSLYSTCLELFKFTIGMGDLE	648
650	FTENYDFKAVFIILLLAYVILTYILLNMLIALMGETVNKIAQESKNIWK	699
649	FTENYDFKAVFIILLLAYVILTYILLNMLIALMGETVNKIAQESKNIWK	698
700	LQRAITILDTEKSFLKCMRKAFRSGKLLQVGYPDGKDDYRWCFRVDEVN	749
699	LQRAITILDTEKSFLKCMRKAFRSGKLLQVGFTPDGKDDYRWCFRVDEVN	748
750	WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSSRVSGRHWKNFALVPLLRE	799
749	WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSGRVSGRNWKNFALVPLLRD	798
800	ASARDRQSAQPEEVYLRQFSGSLKPEDAEOFKSPAASGEK	839
	. : :.	
799	ASTDRRHATQQEEVQLKHYTGSLKPEDAEOFKDSMVPGEK	838

BNSDOCID: <WO_0029577A1_1_>

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CLUSTAL W (1.74) multiple sequence alignment

```

humanVR2.alt -----
humanVR2      MTSPSSSPVFRLETLDGGQEDGSEADRGKLDGSGLPMPMESQFQGEDRKFAPIRVNLNY

humanVR2.alt -----
humanVR2      RKG TGASQPD PNRFD RDR LFN AVSRGV PEDLAGLPEYLSKTSKYLT DSEYTEGSTGKTCL

humanVR2.alt -----
humanVR2      MKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNAQCTDDY YRGHSALHIAIEKRSLQCVK

humanVR2.alt -----GRFFQKGQGTCTFYFGELPLSLAACTKQWDVVSYLENPHQPASLQA
humanVR2      LLVENGANVHARACGRFFQKGQGTCTFYFGELPLSLAACTKQWDVVSYLENPHQPASLQA
                      *****

humanVR2.alt TDSQGN TVLHALVMISDN SAENIALVTS MYDGLLQAGARLCPTVQLEDIRNLQDLTPLKL
humanVR2      TDSQGN TVLHALVMISDN SAENIALVTS MYDGLLQAGARLCPTVQLEDIRNLQDLTPLKL
                      *****

humanVR2.alt AAKEGKIEIFRHILQREFSGLSHLSRKFTWCYGPVVRVSLYDLASVDSCEENSVLEIIAF
humanVR2      AAKEGKIEIFRHILQREFSGLSHLSRKFTWCYGPVVRVSLYDLASVDSCEENSVLEIIAF
                      *****

humanVR2.alt HCKSPHRHRMVVLEPLNKLLOAKWDLLIPKFFDNLFCNLIYMFIFTAVAYHQPTLKKQAA
humanVR2      HCKSPHRHRMVVLEPLNKLLOAKWDLLIPKFFDNLFCNLIYMFIFTAVAYHQPTLKKQAA
                      *****

humanVR2.alt PHLKAEVGN SMLLTG HILIL LGGIYLLVGQLWYFWR RHVFIWISFIDSYFEILFLFQALL
humanVR2      PHLKAEVGN SMLLTG HILIL LGGIYLLVGQLWYFWR RHVFIWISFIDSYFEILFLFQALL
                      *****

humanVR2.alt TVVSQVLCFLAIEWYLP LLVSALVLGWLNL LYYTRGFQHTGIYSVMIQ-----
humanVR2      TVVSQVLCFLAIEWYLP LLVSALVLGWLNL LYYTRGFQHTGIYSVMIQVILRDLLRFL
                      *****

humanVR2.alt -----
humanVR2      IYLVFLPGFAVALVSLSQEAWRPEAPTGP NATESVQPMEGQEDEGNGAQYRGILEASLEL

humanVR2.alt -----
humanVR2      FKFTIGMGELAPQEQLHFRGMV LLLLLAYVLLTYILL LNLIALMSETVNSVATDSWSIW

humanVR2.alt --KKAISVLEMENGYWMCRRKQ RAGVMLTVGTPD GSPDERMCFRVEEVN WASWBQTLPT
humanVR2      KLAISVLEMENGYWMCRRKQ RAGVMLTVGTPD GSPDERMCFRVEEVN WASWBQTLPT
                      :*****

humanVR2.alt LCEDPSGAGVPRTLENPVLASPPKEDEDGASEENYVPVQLLQSN
humanVR2      LCEDPSGAGVPRTLENPVLASPPKEDEDGASEENYVPVQLLQSN
                      *****

```

FIGURE 11

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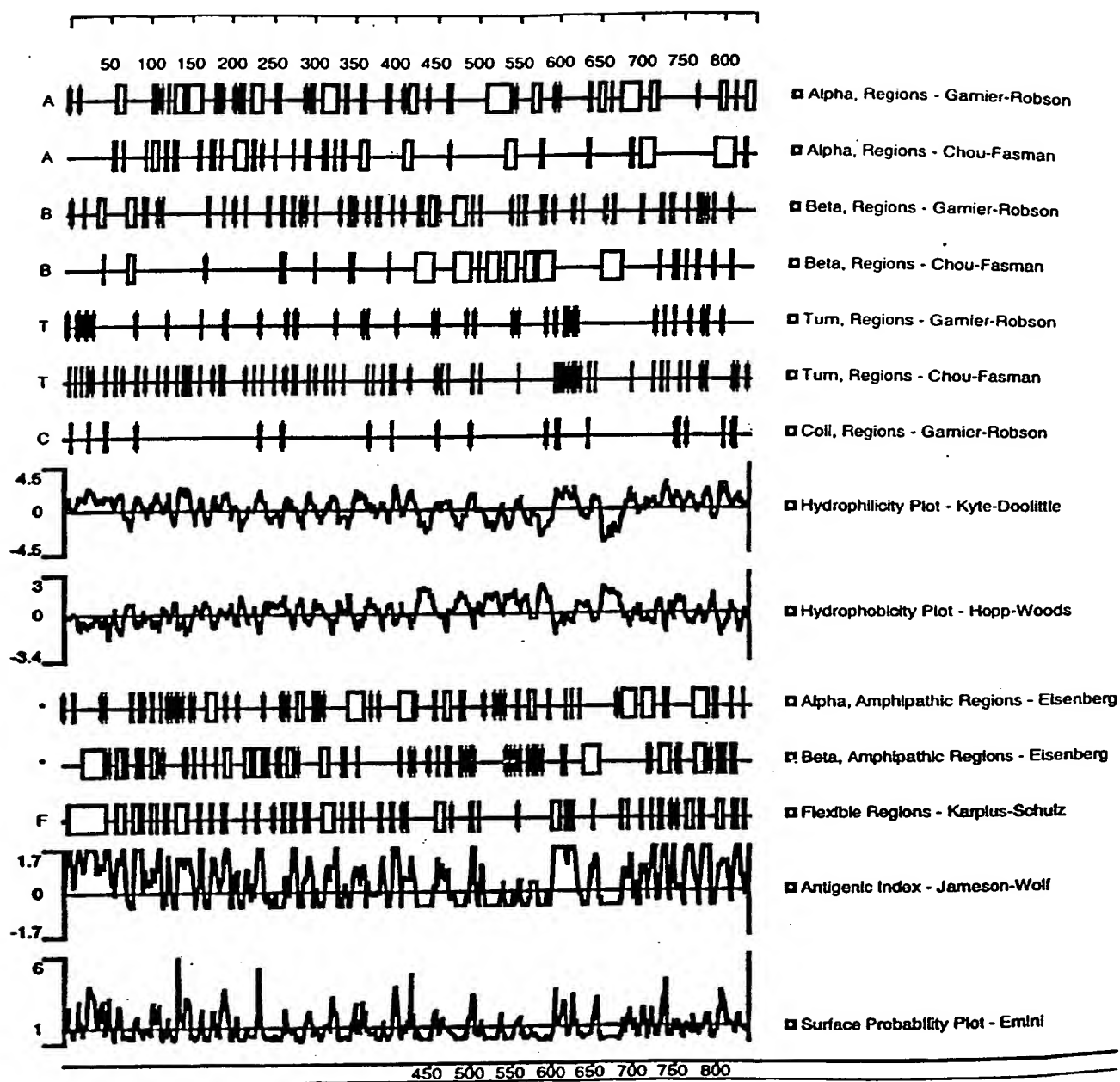


FIGURE 12

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Protein Family / Domain Matches, HMMer version 2

Searching for complete domains

hmmpfam - search a single seq against HMM database

HMMER 2.1.1 (Dec 1998)

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HMMER is freely distributed under the GNU General Public License (GPL).

HMM file: /prod/ddm/seqanal/PFAM/pfam4.2/Pfam
Sequence file: /usr/ns-home/docs/seqanal/orfanal/oa-script.18670.seq

Query: hVR-1

Scores for sequence family classification (score includes all domains):

Model	Description	Score	E-value	N
ank	Ank repeat	51.5	1.9e-11	3

Parsed for domains:

Model	Domain	seq-f	seq-t	hmm-f	hmm-t	score	E-value
ank	1/3	201	233 ..	1	33 []	34.4	2.6e-06
ank	2/3	248	283 ..	1	33 []	13.2	2
ank	3/3	333	361 ..	1	33 []	3.4	26

Alignments of top-scoring domains:

ank: domain 1 of 3, from 201 to 233: score 34.4, E = 2.6e-06

->nGnTPLH1Aarygnvevvk1LLehGAdvnartk<-
+G+T+LH+A + n+ +v lL+e+GAdv a+

hVR-1 201 KGQTALHIAIERRNMALVTLLVENGADVQAAAH 233

ank: domain 2 of 3, from 248 to 283: score 13.2, E = 2

->nGnTPLH1Aarygnvevvk1LLe...hGAdvnartk<-

hVR-1 248 G PL lAa ++++ +vk+LL+++ + Ad+ ar+
FGELPLSLAACTNQLGIVKFLlQnswQTADISARDS 283

ank: domain 3 of 3, from 333 to 361: score 3.4, E = 26

->nGnTPLH1Aarygnvevvk1LLehGAdvnartk<-

hVR-1 333 +G TPL lAa +g++ v ++ L+ ++
KGMTPLALAAGTGKIGVLAYILQ----REIQEP 361

Figure 13

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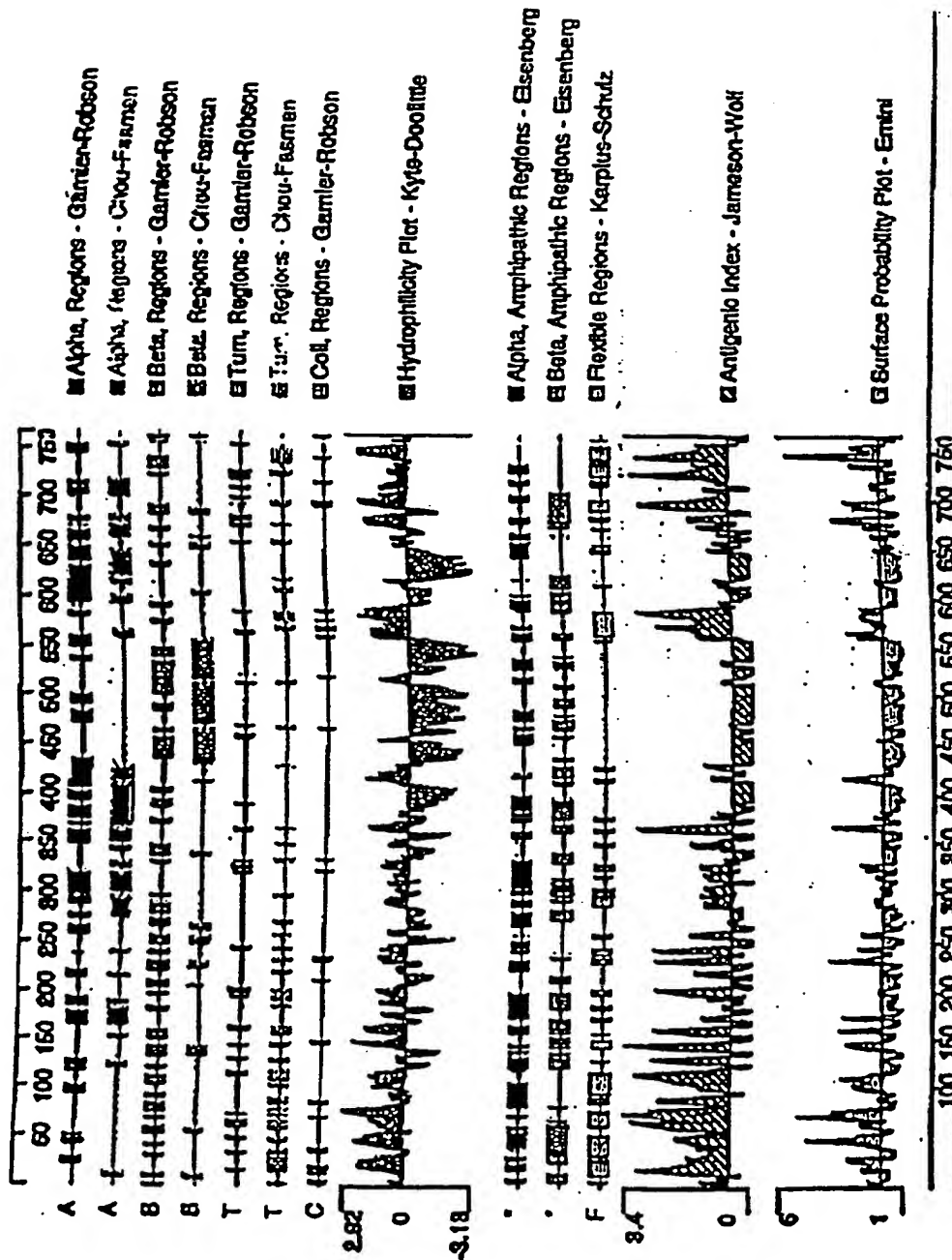


FIGURE 14

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Protein Family / Domain Matches, HMMer version 2

Searching for complete domains

hmmpfam - search a single seq against HMM database

HMMER 2.1.1 (Dec 1998)

Copyright (C) 1992-1998 Washington University School of Medicine

HMMER is freely distributed under the GNU General Public License (GPL).

HMM file: /prod/ddm/seqanal/PFAM/pfam3.3/Pfam
Sequence file: /tmp/orfana1.579.a

Query: Flh21e11

Scores for sequence family classification (score includes all domains):

Model	Description	Score	E-value	N
ank	PF00023 Ank repeat	53.7	4e-12	3

Parsed for domains:

Model	Domain	seq-f	seq-t	hmm-f	hmm-t	score	E-value
ank	1/3	162	194	1	33	38.3	1.7e-07
ank	2/3	208	243	1	33	6.4	4.3
ank	3/3	293	328	1	33	8.8	2.1

Alignments of top-scoring domains:

ank: domain 1 of 3, from 162 to 194: score 38.3, E = 1.7e-07

```

      *-->nGutPLHIAarygnvuvklllehgadvnartk<--
      <G+++LH+A ++ +++++vklle+GAvv+ar
Flh21e11 162  RGRSALHTATEKRSLOQVKLVKRGANVERBAC 194

```

ank: domain 2 of 3, from 208 to 243: score 6.4, E = 4.3

```

      *-->nGutPLHIAarygnvuvklllehgadvnartk<--
      G FL lAn + +++++v +Lle+++ A+ a++
Flh21e11 208  FGELPLSLAARCTKQDVVSVGLSphQPASLOATDS 243

```

ank: domain 3 of 3, from 293 to 328: score 8.8, E = 2.1

```

      *-->nGutPLHIAarygnvuvklllehgadvnartk<--
      + +TFL lAa++g++a+ + l++++ G + +r
Flh21e11 293  QQLATPLSLAAKEOKLETFPHILQracsGLSHLSRK? 328

```

FIGURE 15

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>hvr2.altFL (full-length predicted)
 MTSPTSSSPVFRLETLDDGGQEDGSEADRGKLDGSGLPPMESQFQGEDRKFAPIRVNLNY
 RKG TGASQDPDPNRFDRDRLFNAVSRGVPEDLAGLPEYLSKTSKYLTDSEYTEGSTGKTCL
 MKAVLNLDKGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLOQCVK
 LLVENGANVHARACGRFFQKQGQTCFYFGELPLSLAACTKQWDVVSYLENPHQPASLOATDSQGN TVLHALVM
 ISDNSAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLTPLKLAKEGKIEIFRHIL
 QREFSGLSHLSRKFTWCYGPVRVSLYDLASVDSCEENS VLEIIAFHCKSPHRRHMMVLE
 PLNKLLQAKWDLII PKFFLNFLCNLIYMFIFTAVAYHQPTLKKQAAPHLKAEVGNMMLLT
 GHILILLGGIYLLVGQLWYFWRRHVFIWISFIDSYFEILFLFQALLTVVSQVLCFLAIEW
 YLP LLVSALVLGWLNL LYYTRGFQHTGIYSVMIOKKAI SVLEMENGYWCRKKQORAGVML
 TVG TKPDGSPDERWCFRVEEVNWSWEQTLPTLCEDPGAGVPR TLENPVLASPPKEDED
 GASEENYVPVQLQSN

Figure 16

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CLUSTAL W (1.74) multiple sequence alignment

```

humanVR2      MTSPSSSPVFRLETLDGGQEDGSEADRGKLDGSGGLPPMESQFQGEDRKFPQIRVNLNY
hVR2.altFL    MTSPSSSPVFRLETLDGGQEDGSEADRGKLDGSGGLPPMESQFQGEDRKFPQIRVNLNY
*****

humanVR2      RKG TGASQPD PNR FDR DRLFNAVSRGVPEDLAGLPEYLSKTSKYLT DSEYTEGSTGKTCL
hVR2.altFL    RKG TGASQPD PNR FDR DRLFNAVSRGVPEDLAGLPEYLSKTSKYLT DSEYTEGSTGKTCL
*****

humanVR2      MKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRS LQC VK
hVR2.altFL    MKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRS LQC VK
*****

humanVR2      LLVENGANVHARACGRFFQKGQGTCTFYF GELPLSLAACTKQWDVVS YLLENPHQPASLQA
hVR2.altFL    LLVENGANVHARACGRFFQKGQGTCTFYF GELPLSLAACTKQWDVVS YLLENPHQPASLQA
*****

humanVR2      TDSQGN TVLHALVMISD N SAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLT PLKL
hVR2.altFL    TDSQGN TVLHALVMISD N SAENIALVTSMYDGLLQAGARLCPTVQLEDIRNLQDLT PLKL
*****

humanVR2      AAKEGKIEIFRHILQREFSGLSHLSRKFT EW CYGPVRVSLYDLASVDSCEENSVLEIIAF
hVR2.altFL    AAKEGKIEIFRHILQREFSGLSHLSRKFT EW CYGPVRVSLYDLASVDSCEENSVLEIIAF
*****

humanVR2      HCKSPHRHRMVVLEPLNKL LQAKWDL LIPKFFLNFLCNLIYMFIFTAVAYHQPTLKKQAA
hVR2.altFL    HCKSPHRHRMVVLEPLNKL LQAKWDL LIPKFFLNFLCNLIYMFIFTAVAYHQPTLKKQAA
*****

humanVR2      PHLKAEVGN SMLLTGHILILLGGIYLLVGQLWYFWR RHVFIWISFIDS YFEILFLFQALL
hVR2.altFL    PHLKAEVGN SMLLTGHILILLGGIYLLVGQLWYFWR RHVFIWISFIDS YFEILFLFQALL
*****

humanVR2      TVVSQVLCFLAIEWYLP LLVSALVLGWLNL LYYTRGFQHTGIYSVMIQKVILRDLLRFL
hVR2.altFL    TVVSQVLCFLAIEWYLP LLVSALVLGWLNL LYYTRGFQHTGIYSVMIQK-----
*****

humanVR2      IYLVFLFGFAVALVSLSQEAWRPEAPTGNATESVQPMEGQEDEGNGAQYRGILEASLEL
hVR2.altFL    -----

humanVR2      FKFTIGMGELAFQEQLHFRGMV LLLLLLAYVLLTYILLNMLIALMSETVNSVATDSWSIW
hVR2.altFL    -----

humanVR2      KLQKAISVLEMENGYW WCRKKQ RAGVMLTVGTPDGSPDERWC FRVEEVN WASWEQTLPT
hVR2.altFL    ---KAISVLEMENGYW WCRKKQ RAGVMLTVGTPDGSPDERWC FRVEEVN WASWEQTLPT
*****

humanVR2      LCEDPSGAGVPRTLENPVLASPPKEDEDGASEENYVPVQLLQSN
hVR2.altFL    LCEDPSGAGVPRTLENPVLASPPKEDEDGASEENYVPVQLLQSN
*****

```

Figure 17

- 1 -

SEQUENCE LISTING

<110> MILLENNIUM PHARMACEUTICALS, INC.

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FAMILY OF PROTEINS AND USES THEREOF

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<150> 60/114,078

<151> 1998-12-28

<150> 09/258,633

<151> 1999-02-26

<150> 09/421,134

<151> 1999-10-19

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<220>

<221> CDS

<222> (1113)..(3629)

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- 3 -

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Asp Gly Gln Asn Thr Thr Ile Pro Leu Leu Leu Glu Ile Ala Arg Gln	
170 175 180	
acg gac agc ctg aag gag ctt gtc aac gcc agc tac acg gac agc tac	1709
Thr Asp Ser Leu Lys Glu Leu Val Asn Ala Ser Tyr Thr Asp Ser Tyr	
185 190 195	
tac aag ggc cag aca gca ctg cac atc gcc atc gag aga cgc aac atg	1757
Tyr Lys Gly Gln Thr Ala Leu His Ile Ala Ile Glu Arg Arg Asn Met	
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gcc ctg gtg acc ctc ctg gtg gag aac gga gca gac gtc cag gct gcg	1805
Ala Leu Val Thr Leu Leu Val Glu Asn Gly Ala Asp Val Gln Ala Ala	
220 225 230	
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Ala His Gly Asp Phe Phe Lys Lys Thr Lys Gly Arg Pro Gly Phe Tyr	
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ttc ggt gaa ctg ccc ctg tcc ctg gcc gcg tgc acc aac cag ctg ggc	1901
Phe Gly Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Asn Gln Leu Gly	
250 255 260	
atc gtg aag ttc ctg ctg cag aac tcc tgg cag acg gcc gac atc agc	1949
Ile Val Lys Phe Leu Leu Gln Asn Ser Trp Gln Thr Ala Asp Ile Ser	
265 270 275	
gcc agg gac tcg gtg ggc aac acg gtg ctg cac gcc ctg gtg gag gtg	1997
Ala Arg Asp Ser Val Gly Asn Thr Val Leu His Ala Leu Val Glu Val	
280 285 290 295	
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315 320 325	
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Glu Leu Thr Asn Lys Lys Gly Met Thr Pro Leu Ala Leu Ala Ala Gly	
330 335 340	
acc ggg aag atc ggg gtc ttg gcc tat att ctc cag cgg gag atc cag	2189
Thr Gly Lys Ile Gly Val Leu Ala Tyr Ile Leu Gln Arg Glu Ile Gln	
345 350 355	
gag ccc gag tgc agg cac ctg tcc agg aag ttc acc gag tgg gcc tac	2237
Glu Pro Glu Cys Arg His Leu Ser Arg Lys Phe Thr Glu Trp Ala Tyr	
360 365 370 375	
ggg ccc gtg cac tcc tcg ctg tac gac ctg tcc tgc atc gac acc tgc	2285
Gly Pro Val His Ser Ser Leu Tyr Asp Leu Ser Cys Ile Asp Thr Cys	
380 385 390	
gag aag aac tcg gtg ctg gag gtg atc gcc tac agc agc agc gag acc	2333
Glu Lys Asn Ser Val Leu Glu Val Ile Ala Tyr Ser Ser Ser Glu Thr	

- 4 -

395	400	405	
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Pro Asn Arg His Asp Met Leu Leu Val Glu Pro Leu Asn Arg Leu Leu			
410	415	420	
cag gac aag tgg gac aga ttc gtc aag cgc atc ttc tac ttc aac ttc			2429
Gln Asp Lys Trp Asp Arg Phe Val Lys Arg Ile Phe Tyr Phe Asn Phe			
425	430	435	
ctg gtc tac tgc ctg tac atg atc atc ttc acc atg gct gcc tac tac			2477
Leu Val Tyr Cys Leu Tyr Met Ile Ile Phe Thr Met Ala Ala Tyr Tyr			
440	445	450	455
agg ccc gtg gat ggc ttg cct ccc ttt aag atg gaa aaa att gga gac			2525
Arg Pro Val Asp Gly Leu Pro Pro Phe Lys Met Glu Lys Ile Gly Asp			
460	465	470	
tat ttc cga gtt act gga gag atc ctg tct gtg tta gga gga gtc tac			2573
Tyr Phe Arg Val Thr Gly Glu Ile Leu Ser Val Leu Gly Gly Val Tyr			
475	480	485	
ttc ttt ttc cga ggg att cag tat ttc ctg cag agg cgg ccg tcg atg			2621
Phe Phe Phe Arg Gly Ile Gln Tyr Phe Leu Gln Arg Arg Pro Ser Met			
490	495	500	
aag acc ctg ttt gtg gac agc tac agt gag atg ctt ttc ttt ctg cag			2669
Lys Thr Leu Phe Val Asp Ser Tyr Ser Glu Met Leu Phe Phe Leu Gln			
505	510	515	
tca ctg ttc atg ctg gcc acc gtg gtg ctg tac ttc agc cac ctc aag			2717
Ser Leu Phe Met Leu Ala Thr Val Val Leu Tyr Phe Ser His Leu Lys			
520	525	530	535
gag tat gtg gct tcc atg gta ttc tcc ctg gcc ttg ggc tgg acc aac			2765
Glu Tyr Val Ala Ser Met Val Phe Ser Leu Ala Leu Gly Trp Thr Asn			
540	545	550	
atg ctc tac tac acc cgc ggt ttc cag cag atg ggc atc tat gcc gtc			2813
Met Leu Tyr Tyr Thr Arg Gly Phe Gln Gln Met Gly Ile Tyr Ala Val			
555	560	565	
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570	575	580	
tac atc gtc ttc ttg ttc ggg ttt tcc aca gcg gtg gtg acg ctg att			2909
Tyr Ile Val Phe Leu Phe Gly Phe Ser Thr Ala Val Val Thr Leu Ile			
585	590	595	
gaa gac ggg aag aat gac tcc ctg ccg tct gag tcc acg tcg cac agg			2957
Glu Asp Gly Lys Asn Asp Ser Leu Pro Ser Glu Ser Thr Ser His Arg			
600	605	610	615
tgg cgg ggg cct gcc tgc agg ccc ccc gat agc tcc tac aac agc ctg			3005
Trp Arg Gly Pro Ala Cys Arg Pro Pro Asp Ser Ser Tyr Asn Ser Leu			
620	625	630	

- 5 -

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Tyr Ser Thr Cys Leu Glu Leu Phe Lys Phe Thr Ile Gly Met Gly Asp	
635 640 645	
ctg gag ttc act gag aac tat gac ttc aag gct gtc ttc atc atc ctg	3101
Leu Glu Phe Thr Glu Asn Tyr Asp Phe Lys Ala Val Phe Ile Ile Leu	
650 655 660	
ctg ctg gcc tat gta att ctc acc tac atc ctc ctg ctc aac atg ctc	3149
Leu Leu Ala Tyr Val Ile Leu Thr Tyr Ile Leu Leu Leu Asn Met Leu	
665 670 675	
atc gcc ctc atg ggt gag act gtc aac aag atc gca cag gag agc aag	3197
Ile Ala Leu Met Gly Glu Thr Val Asn Lys Ile Ala Gln Glu Ser Lys	
680 685 690 695	
aac atc tgg aag ctg cag aga gcc atc acc atc ctg gac acg gag aag	3245
Asn Ile Trp Lys Leu Gln Arg Ala Ile Thr Ile Leu Asp Thr Glu Lys	
700 705 710	
agc ttc ctt aag tgc atg agg aag gcc ttc cgc tca ggc aag ctg ctg	3293
Ser Phe Leu Lys Cys Met Arg Lys Ala Phe Arg Ser Gly Lys Leu Leu	
715 720 725	
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Gln Val Gly Tyr Thr Pro Asp Gly Lys Asp Asp Tyr Arg Trp Cys Phe	
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agg gtg gac gag gtg aac tgg acc acc tgg aac acc aac gtg ggc atc	3389
Arg Val Asp Glu Val Asn Trp Thr Thr Trp Asn Thr Asn Val Gly Ile	
745 750 755	
atc aac gaa gac ccg ggc aac tgt gag ggc gtc aag cgc acc ctg agc	3437
Ile Asn Glu Asp Pro Gly Asn Cys Glu Gly Val Lys Arg Thr Leu Ser	
760 765 770 775	
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Phe Ser Leu Arg Ser Ser Arg Val Ser Gly Arg His Trp Lys Asn Phe	
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gcc ctg gtc ccc ctt tta aga gag gca agt gct cga gat agg cag tct	3533
Ala Leu Val Pro Leu Leu Arg Glu Ala Ser Ala Arg Asp Arg Gln Ser	
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gct cag ccc gag gaa gtt tat ctg cga cag ttt tca ggg tct ctg aag	3581
Ala Gln Pro Glu Glu Val Tyr Leu Arg Gln Phe Ser Gly Ser Leu Lys	
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cca gag gac gct gag gtc ttc aag agt cct gcc gct tcc ggg gag aag	3629
Pro Glu Asp Ala Glu Val Phe Lys Ser Pro Ala Ala Ser Gly Glu Lys	
825 830 835	
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 Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Phe Pro Val Asp Cys Pro
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 His Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro
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 Val Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu
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 Tyr Asp Arg Arg Ser Ile Phe Glu Ala Val Ala Gln Asn Asn Cys Gln
 115 120 125
 Asp Leu Glu Ser Leu Leu Leu Phe Leu Gln Lys Ser Lys Lys His Leu
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 Lys Ala Met Leu Asn Leu His Asp Gly Gln Asn Thr Thr Ile Pro Leu
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 Leu Leu Glu Ile Ala Arg Gln Thr Asp Ser Leu Lys Glu Leu Val Asn
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 Ala Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile
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 Ala Ile Glu Arg Arg Asn Met Ala Leu Val Thr Leu Leu Val Glu Asn
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Gly Ala Asp Val Gln Ala Ala Ala His Gly Asp Phe Phe Lys Lys Thr
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 Lys Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala
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 Ala Cys Thr Asn Gln Leu Gly Ile Val Lys Phe Leu Leu Gln Asn Ser
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 Trp Gln Thr Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val
 275 280 285
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 Phe Val Thr Ser Met Tyr Asn Glu Ile Leu Met Leu Gly Ala Lys Leu
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 His Pro Thr Leu Lys Leu Glu Glu Leu Thr Asn Lys Lys Gly Met Thr
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 Ile Leu Gln Arg Glu Ile Gln Glu Pro Glu Cys Arg His Leu Ser Arg
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 Lys Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp
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 Lys Met Glu Lys Ile Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu
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 Ser Val Leu Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe
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 Leu Gln Arg Arg Pro Ser Met Lys Thr Leu Phe Val Asp Ser Tyr Ser
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 Glu Met Leu Phe Phe Leu Gln Ser Leu Phe Met Leu Ala Thr Val Val
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 Leu Tyr Phe Ser His Leu Lys Glu Tyr Val Ala Ser Met Val Phe Ser

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Gln 565	Met Gly Ile Tyr	Ala Val Met Ile	Glu 570 Lys Met Ile Leu Arg Asp 575	
Leu 580	Cys Arg Phe Met	Phe Val Tyr Ile	Val 585 Phe Leu Phe Gly Phe Ser 590	
Thr 595	Ala Val Val Thr	Leu Ile Glu 600	Asp Gly Lys Asn Asp 605	Ser Leu Pro
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Asp 625	Ser Ser Tyr Asn	Ser Leu Tyr Ser	Thr 630 Cys Leu Glu Leu Phe Lys 640	
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Lys 690	Ile Ala Gln Glu	Ser Lys Asn Ile	Trp 695 Lys Leu Gln Arg Ala Ile 700	
Thr 705	Ile Leu Asp Thr	Glu Lys Ser Phe	Leu 710 Lys Cys Met Arg Lys Ala 720	
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Gly 785	Arg His Trp Lys	Asn Phe Ala Leu	Val 790 Pro Leu Leu Arg Glu Ala 800	
Ser 805	Ala Arg Asp Arg	Gln Ser Ala Gln	Pro 810 Glu Glu Val Tyr Leu Arg 815	
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caa aag gac acc tgc cca gac ccc ctg gat gga gac cct aac tcc agg 96
 Gln Lys Asp Thr Cys Pro Asp Pro Leu Asp Gly Asp Pro Asn Ser Arg
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cca cct cca gcc aag ccc cag ctc ccc acg gcc aag agc cgc acc cgg 144
 Pro Pro Pro Ala Lys Pro Gln Leu Pro Thr Ala Lys Ser Arg Thr Arg
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ctc ttt ggg aag ggt gac tgc gag gag gct ttc ccg gtg gat tgc ccc 192
 Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Phe Pro Val Asp Cys Pro
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cac gag gaa ggt gag ttg gac tcc tgc ccg acc atc aca gtc agc cct 240
 His Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro
 65 70 75 80

gtt atc acc atc cag agg cca gga gac ggc ccc acc ggt gcc agg ctg 288
 Val Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu
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 Leu Ser Gln Asp Ser Val Ala Ala Ser Thr Glu Lys Thr Leu Arg Leu
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 Tyr Asp Arg Arg Ser Ile Phe Glu Ala Val Ala Gln Asn Asn Cys Gln
 115 120 125

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 Asp Leu Glu Ser Leu Leu Phe Leu Gln Lys Ser Lys Lys His Leu
 130 135 140

aca gac aac gag ttc aaa gac cct gag aca ggg aag acc tgt ctg ctg 480
 Thr Asp Asn Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu
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 Lys Ala Met Leu Asn Leu His Asp Gly Gln Asn Thr Thr Ile Pro Leu
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ctc ctg gag atc gcg cgg caa acg gac agc ctg aag gag ctt gtc aac 576

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Leu	Leu	Glu	Ile	Ala	Arg	Gln	Thr	Asp	Ser	Leu	Lys	Glu	Leu	Val	Asn	
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gcc	agc	tac	acg	gac	agc	tac	tac	aag	ggc	cag	aca	gca	ctg	cac	atc	624
Ala	Ser	Tyr	Thr	Asp	Ser	Tyr	Tyr	Lys	Gly	Gln	Thr	Ala	Leu	His	Ile	
		195					200					205				
gcc	atc	gag	aga	cgc	aac	atg	gcc	ctg	gtg	acc	ctc	ctg	gtg	gag	aac	672
Ala	Ile	Glu	Arg	Arg	Asn	Met	Ala	Leu	Val	Thr	Leu	Leu	Val	Glu	Asn	
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Lys	Gly	Arg	Pro	Gly	Phe	Tyr	Phe	Gly	Glu	Leu	Pro	Leu	Ser	Leu	Ala	
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gcg	tgc	acc	aac	cag	ctg	ggc	atc	gtg	aag	ttc	ctg	ctg	cag	aac	tcc	816
Ala	Cys	Thr	Asn	Gln	Leu	Gly	Ile	Val	Lys	Phe	Leu	Leu	Gln	Asn	Ser	
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Trp	Gln	Thr	Ala	Asp	Ile	Ser	Ala	Arg	Asp	Ser	Val	Gly	Asn	Thr	Val	
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Leu	His	Ala	Leu	Val	Glu	Val	Ala	Asp	Asn	Thr	Ala	Asp	Asn	Thr	Lys	
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Phe	Val	Thr	Ser	Met	Tyr	Asn	Glu	Ile	Leu	Met	Leu	Gly	Ala	Lys	Leu	
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His	Pro	Thr	Leu	Lys	Leu	Glu	Glu	Leu	Thr	Asn	Lys	Lys	Gly	Met	Thr	
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ccg	ctg	gct	ctg	gca	gct	ggg	acc	ggg	aag	atc	ggg	gtc	ttg	gcc	tat	1056
Pro	Leu	Ala	Leu	Ala	Ala	Gly	Thr	Gly	Lys	Ile	Gly	Val	Leu	Ala	Tyr	
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Ile	Leu	Gln	Arg	Glu	Ile	Gln	Glu	Pro	Glu	Cys	Arg	His	Leu	Ser	Arg	
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aag	ttc	acc	gag	tgg	gcc	tac	ggg	ccc	gtg	cac	tcc	tgc	ctg	tac	gac	1152
Lys	Phe	Thr	Glu	Trp	Ala	Tyr	Gly	Pro	Val	His	Ser	Ser	Leu	Tyr	Asp	
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ctg	tcc	tgc	atc	gac	acc	tgc	gag	aag	aac	tgc	gtg	ctg	gag	gtg	atc	1200
Leu	Ser	Cys	Ile	Asp	Thr	Cys	Glu	Lys	Asn	Ser	Val	Leu	Glu	Val	Ile	
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gcc	tac	agc	agc	agc	gag	acc	cct	aat	cgc	cac	gac	atg	ctc	ttg	gtg	1248
Ala	Tyr	Ser	Ser	Ser	Glu	Thr	Pro	Asn	Arg	His	Asp	Met	Leu	Leu	Val	
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Arg Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile	
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ttc acc atg gct gcc tac tac agg ccc gtg gat ggc ttg cct ccc ttt	1392
Phe Thr Met Ala Ala Tyr Tyr Arg Pro Val Asp Gly Leu Pro Pro Phe	
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Lys Met Glu Lys Ile Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu	
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Ser Val Leu Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe	
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Glu Met Leu Phe Phe Leu Gln Ser Leu Phe Met Leu Ala Thr Val Val	
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Leu Ala Leu Gly Trp Thr Asn Met Leu Tyr Tyr Thr Arg Gly Phe Gln	
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Gln Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp	
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Asp Ser Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys	
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Lys Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr	
660 665 670	
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Lys Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile	
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acc atc ctg gac acg gag aag agc ttc ctt aag tgc atg agg aag gcc	2160
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Asp Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr	
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Trp Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu	
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Gly Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser	
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785 790 795 800	
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Tyr Leu Ser Lys Thr Ser Lys Tyr Leu Thr Asp Ser Glu Tyr Thr Glu
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Gly	Glu	Leu	Pro	Leu	Ser	Leu	Ala	Ala	Cys	Thr	Lys	Gln	Trp	Asp	Val														
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Ile	Arg	Asn	Leu	Gln	Asp	Leu	Thr	Pro	Leu	Lys	Leu	Ala	Ala	Lys	Glu														
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Gly	Lys	Ile	Glu	Ile	Phe	Arg	His	Ile	Leu	Gln	Arg	Glu	Phe	Ser	Gly														
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Arg	Val	Ser	Leu	Tyr	Asp	Leu	Ala	Ser	Val	Asp	Ser	Cys	Glu	Glu	Asn														
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tca	gtg	ctg	gag	atc	att	gcc	ttt	cat	tgc	aag	agc	cgc	cac	cga	cac														
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cga	atg	gtc	gtt	ttg	gag	ccc	ctg	aac	aaa	ctg	ctg	cag	gcg	aaa	tgg														
Arg	Met	Val	Val	Leu	Glu	Pro	Leu	Asn	Lys	Leu	Leu	Gln	Ala	Lys	Trp														
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gat	ctg	ctc	atc	ccc	aag	ttc	ttc	tta	aac	ttc	ctg	tgt	aat	ctg	atc														
Asp	Leu	Leu	Ile	Pro	Lys	Phe	Phe	Leu	Asn	Phe	Leu	Cys	Asn	Leu	Ile														
					390						395																		

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Tyr Met Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys	
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Lys Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu	
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ctg acg ggc cac atc ctt atc ctg cta ggg ggg atc tac ctc ctc gtg	1704
Leu Thr Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val	
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Gly Gln Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Ser	
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Phe Ile Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu	
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Thr Val Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu	
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ccc ctg ctt gtg tct gcg ctg gtg ctg ggc tgg ctg aac ctg ctt tac	1896
Pro Leu Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr	
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Tyr Thr Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln	
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Lys Val Ile Leu Arg Asp Leu Leu Arg Phe Leu Leu Ile Tyr Leu Val	
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Phe Leu Phe Gly Phe Ala Val Ala Leu Val Ser Leu Ser Gln Glu Ala	
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Trp Arg Pro Glu Ala Pro Thr Gly Pro Asn Ala Thr Glu Ser Val Gln	
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Pro Met Glu Gly Gln Glu Asp Glu Gly Asn Gly Ala Gln Tyr Arg Gly	
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Ile Leu Glu Ala Ser Leu Glu Leu Phe Lys Phe Thr Ile Gly Met Gly	
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gag ctg gcc ttc cag gag cag ctg cac ttc cgc ggc atg gtg ctg ctg	2232
Glu Leu Ala Phe Gln Glu Gln Leu His Phe Arg Gly Met Val Leu Leu	
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ctg ctg ctg gcc tac gtg ctg ctc acc tac atc ctg ctg ctc aac atg	2280

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Leu Leu Leu Ala Tyr Val Leu Leu Thr Tyr Ile Leu Leu Leu Asn Met
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ctc atc gcc ctc atg agc gag acc gtc aac agt gtc gcc act gac agc 2328
Leu Ile Ala Leu Met Ser Glu Thr Val Asn Ser Val Ala Thr Asp Ser
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tgg agc atc tgg aag ctg cag aaa gcc atc tct gtc ctg gag atg gag 2376
Trp Ser Ile Trp Lys Leu Gln Lys Ala Ile Ser Val Leu Glu Met Glu
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aat ggc tat tgg tgg tgc agg aag aag cag cgg gca ggt gtg atg ctg 2424
Asn Gly Tyr Trp Trp Cys Arg Lys Lys Gln Arg Ala Gly Val Met Leu
675                               680                               685

acc gtt ggc act aag cca gat ggc agc ccg gat gag cgc tgg tgc ttc 2472
Thr Val Gly Thr Lys Pro Asp Gly Ser Pro Asp Glu Arg Trp Cys Phe
690                               695                               700

agg gtg gag gag gtg aac tgg gct tca tgg gag cag acg ctg cct acg 2520
Arg Val Glu Glu Val Asn Trp Ala Ser Trp Glu Gln Thr Leu Pro Thr
705                               710                               715                               720

ctg tgt gag gac ccg tca ggg gca ggt gtc cct cga act ctc gag aac 2568
Leu Cys Glu Asp Pro Ser Gly Ala Gly Val Pro Arg Thr Leu Glu Asn
725                               730                               735

cct gtc ctg gct tcc cct ccc aag gag gat gag gat ggt gcc tct gag 2616
Pro Val Leu Ala Ser Pro Pro Lys Glu Asp Glu Asp Gly Ala Ser Glu
740                               745                               750

gaa aac tat gtg ccc gtc cag ctc ctc cag tcc aac tgatggccca 2662
Glu Asn Tyr Val Pro Val Gln Leu Leu Gln Ser Asn
755                               760

gatgcagcag gaggccagag gacagagcag aggatctttc caaccacatc tgctggctct 2722

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Lys Phe Ala Pro Gln Ile Arg Val Asn Leu Asn Tyr Arg Lys Gly Thr
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 Asn Ala Val Ser Arg Gly Val Pro Glu Asp Leu Ala Gly Leu Pro Glu
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 Gly Ser Thr Gly Lys Thr Cys Leu Met Lys Ala Val Leu Asn Leu Lys
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 Ser Gly Asn Pro Gln Pro Leu Val Asn Ala Gln Cys Thr Asp Asp Tyr
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 Tyr Arg Gly His Ser Ala Leu His Ile Ala Ile Glu Lys Arg Ser Leu
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 Gln Cys Val Lys Leu Leu Val Glu Asn Gly Ala Asn Val His Ala Arg
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 Ala Cys Gly Arg Phe Phe Gln Lys Gly Gln Gly Thr Cys Phe Tyr Phe
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 Gly Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val
 210 215 220
 Val Ser Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala
 225 230 235 240
 Thr Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser
 245 250 255
 Asp Asn Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly
 260 265 270
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 Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly
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 325 330 335
 Arg Val Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn
 340 345 350
 Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His
 355 360 365

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Arg Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp
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 Asp Leu Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile
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 Tyr Met Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys
 405 410 415
 Lys Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu
 420 425 430
 Leu Thr Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val
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 Gly Gln Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Ser
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 Phe Ile Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu
 465 470 475 480
 Thr Val Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu
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 Pro Leu Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr
 500 505 510
 Tyr Thr Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln
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 Lys Val Ile Leu Arg Asp Leu Leu Arg Phe Leu Leu Ile Tyr Leu Val
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 Phe Leu Phe Gly Phe Ala Val Ala Leu Val Ser Leu Ser Gln Glu Ala
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 Trp Arg Pro Glu Ala Pro Thr Gly Pro Asn Ala Thr Glu Ser Val Gln
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 Pro Met Glu Gly Gln Glu Asp Glu Gly Asn Gly Ala Gln Tyr Arg Gly
 580 585 590
 Ile Leu Glu Ala Ser Leu Glu Leu Phe Lys Phe Thr Ile Gly Met Gly
 595 600 605
 Glu Leu Ala Phe Gln Glu Gln Leu His Phe Arg Gly Met Val Leu Leu
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 Leu Leu Leu Ala Tyr Val Leu Leu Thr Tyr Ile Leu Leu Leu Asn Met
 625 630 635 640
 Leu Ile Ala Leu Met Ser Glu Thr Val Asn Ser Val Ala Thr Asp Ser
 645 650 655
 Trp Ser Ile Trp Lys Leu Gln Lys Ala Ile Ser Val Leu Glu Met Glu
 660 665 670
 Asn Gly Tyr Trp Trp Cys Arg Lys Lys Gln Arg Ala Gly Val Met Leu
 675 680 685

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Thr Val Gly Thr Lys Pro Asp Gly Ser Pro Asp Glu Arg Trp Cys Phe
690 695 700

Arg Val Glu Glu Val Asn Trp Ala Ser Trp Glu Gln Thr Leu Pro Thr
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Leu Cys Glu Asp Pro Ser Gly Ala Gly Val Pro Arg Thr Leu Glu Asn
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Gly Gly Gln Glu Asp Gly Ser Glu Ala Asp Arg Gly Lys Leu Asp Phe
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ggg agc ggg ctg cct ccc atg gag tca cag ttc cag ggc gag gac cgg 144
Gly Ser Gly Leu Pro Pro Met Glu Ser Gln Phe Gln Gly Glu Asp Arg
35 40 45

aaa ttc gcc cct cag ata aga gtc aac ctc aac tac cga aag gga aca 192
Lys Phe Ala Pro Gln Ile Arg Val Asn Leu Asn Tyr Arg Lys Gly Thr
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ggt gcc agt cag ccg gat cca aac cga ttt gac cga gat cgg ctc ttc 240
Gly Ala Ser Gln Pro Asp Pro Asn Arg Phe Asp Arg Asp Arg Leu Phe
65 70 75 80

aat gcg gtc tcc cgg ggt gtc ccc gag gat ctg gct gga ctt cca gag 288
Asn Ala Val Ser Arg Gly Val Pro Glu Asp Leu Ala Gly Leu Pro Glu
85 90 95

tac ctg agc aag acc agc aag tac ctc acc gac tcg gaa tac aca gag 336
Tyr Leu Ser Lys Thr Ser Lys Tyr Leu Thr Asp Ser Glu Tyr Thr Glu
100 105 110

ggc tcc aca ggt aag acg tgc ctg atg aag gct gtg ctg aac ctt aag 384
Gly Ser Thr Gly Lys Thr Cys Leu Met Lys Ala Val Leu Asn Leu Lys

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tct ggc aat cct cag ccc ctg gta aat gcc cag tgc aca gat gac tat Ser Gly Asn Pro Gln Pro Leu Val Asn Ala Gln Cys Thr Asp Asp Tyr 145 150 155 160			480
tac cga ggc cac agc gct ctg cac atc gcc att gag aag agg agt ctg Tyr Arg Gly His Ser Ala Leu His Ile Ala Ile Glu Lys Arg Ser Leu 165 170 175			528
cag tgt gtg aag ctc ctg gtg gag aat ggg gcc aat gtg cat gcc cgg Gln Cys Val Lys Leu Leu Val Glu Asn Gly Ala Asn Val His Ala Arg 180 185 190			576
gcc tgc ggc cgc ttc ttc cag aag ggc caa ggg act tgc ttt tat ttc Ala Cys Gly Arg Phe Phe Gln Lys Gly Gln Gly Thr Cys Phe Tyr Phe 195 200 205			624
ggc gag cta ccc ctc tct ttg gcc gct tgc acc aag cag tgg gat gtg Gly Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val 210 215 220			672
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act gac tcc cag ggc aac aca gtc ctg cat gcc cta gtg atg atc tcg Thr Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser 245 250 255			768
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Arg	Met	Val	Val	Leu	Glu	Pro	Leu	Asn	Lys	Leu	Leu	Gln	Ala	Lys	Trp	
	370					375					380					
gat	ctg	ctc	atc	ccc	aag	ttc	ttc	tta	aac	ttc	ctg	tgt	aat	ctg	atc	1200
Asp	Leu	Leu	Ile	Pro	Lys	Phe	Phe	Leu	Asn	Phe	Leu	Cys	Asn	Leu	Ile	
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Tyr	Met	Phe	Ile	Phe	Thr	Ala	Val	Ala	Tyr	His	Gln	Pro	Thr	Leu	Lys	
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Lys	Gln	Ala	Ala	Pro	His	Leu	Lys	Ala	Glu	Val	Gly	Asn	Ser	Met	Leu	
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ctg	acg	ggc	cac	atc	ctt	atc	ctg	cta	ggg	ggg	atc	tac	ctc	ctc	gtg	1344
Leu	Thr	Gly	His	Ile	Leu	Ile	Leu	Leu	Gly	Gly	Ile	Tyr	Leu	Leu	Val	
		435					440					445				
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Gly	Gln	Leu	Trp	Tyr	Phe	Trp	Arg	Arg	His	Val	Phe	Ile	Trp	Ile	Ser	
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Phe	Ile	Asp	Ser	Tyr	Phe	Glu	Ile	Leu	Phe	Leu	Phe	Gln	Ala	Leu	Leu	
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Thr	Val	Val	Ser	Gln	Val	Leu	Cys	Phe	Leu	Ala	Ile	Glu	Trp	Tyr	Leu	
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Pro	Leu	Leu	Val	Ser	Ala	Leu	Val	Leu	Gly	Trp	Leu	Asn	Leu	Leu	Tyr	
			500					505					510			
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Tyr	Thr	Arg	Gly	Phe	Gln	His	Thr	Gly	Ile	Tyr	Ser	Val	Met	Ile	Gln	
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aag	gtc	atc	ctg	cgg	gac	ctg	ctg	cgc	ttc	ctt	ctg	atc	tac	tta	gtc	1632
Lys	Val	Ile	Leu	Arg	Asp	Leu	Leu	Arg	Phe	Leu	Leu	Ile	Tyr	Leu	Val	
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Phe	Leu	Phe	Gly	Phe	Ala	Val	Ala	Leu	Val	Ser	Leu	Ser	Gln	Glu	Ala	
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Trp	Arg	Pro	Glu	Ala	Pro	Thr	Gly	Pro	Asn	Ala	Thr	Glu	Ser	Val	Gln	
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Ser Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala Thr	
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gac tcc cag ggc aac aca gtc ctg cat gcc cta gtg atg atc tcg gac	191
Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser Asp	
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Asn Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly Leu	
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Leu Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp Ile	
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Val Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn Ser	
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Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His Arg	
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Met Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys Lys	
210 215 220	
cag gcc gcc cct cac ctg aaa gcg gag gtt gga aac tcc atg ctg ctg	719
Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu Leu	

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225	230	235	
acg ggc cac atc ctt	atc ctg cta ggg ggg	atc tac ctc ctc gtg ggc	767
Thr Gly His Ile Leu	Ile Leu Leu Gly Gly	Ile Tyr Leu Leu Val Gly	
240	245	250	255
cag ctg tgg tac ttc	tgg cgg cgc cac gtg	ttc atc tgg atc tgc ttc	815
Gln Leu Trp Tyr Phe	Trp Arg Arg His Val	Phe Ile Trp Ile Ser Phe	
	260	265	270
ata gac agc tac ttt	gaa atc ctc ttc ctg	ttc cag gcc ctg ctc aca	863
Ile Asp Ser Tyr Phe	Glu Ile Leu Phe Leu	Phe Gln Ala Leu Leu Thr	
	275	280	285
gtg gtg tcc cag gtg	ctg tgt ttc ctg gcc	atc gag tgg tac ctg ccc	911
Val Val Ser Gln Val	Leu Cys Phe Leu Ala	Ile Glu Trp Tyr Leu Pro	
	290	295	300
ctg ctt gtg tct gcg	ctg gtg ctg ggc tgg	ctg aac ctg ctt tac tat	959
Leu Leu Val Ser Ala	Leu Val Leu Gly Trp	Leu Asn Leu Leu Tyr Tyr	
	305	310	315
aca cgt ggc ttc cag	cac aca ggc atc tac	agt gtc atg atc cag aag	1007
Thr Arg Gly Phe Gln	His Thr Gly Ile Tyr	Ser Val Met Ile Gln Lys	
	325	330	335
aaa gcc atc tct gtc	ctg gag atg gag aat	ggc tat tgg tgg tgc agg	1055
Lys Ala Ile Ser Val	Leu Glu Met Glu Asn	Gly Tyr Trp Trp Cys Arg	
	340	345	350
aag aag cag cgg gca	ggt gtg atg ctg acc	gtt ggc act aag cca gat	1103
Lys Lys Gln Arg Ala	Gly Val Met Leu Thr	Val Gly Thr Lys Pro Asp	
	355	360	365
ggc agc ccg gat gag	cgc tgg tgc ttc agg	gtg gag gag gtg aac tgg	1151
Gly Ser Pro Asp Glu	Arg Trp Cys Phe Arg	Val Glu Glu Val Asn Trp	
	370	375	380
gct tca tgg gag cag	acg ctg cct acg ctg	tgt gag gac ccg tca ggg	1199
Ala Ser Trp Glu Gln	Thr Leu Pro Thr Leu	Cys Glu Asp Pro Ser Gly	
	385	390	395
gca ggt gtc cct cga	act ctc gag aac cct	gtc ctg gct tcc cct ccc	1247
Ala Gly Val Pro Arg	Thr Leu Glu Asn Pro	Val Leu Ala Ser Pro Pro	
	400	405	410
aag gag gat gag gat	ggt gcc tct gag gaa	aac tat gtg ccc gtc cag	1295
Lys Glu Asp Glu Asp	Gly Ala Ser Glu Glu	Asn Tyr Val Pro Val Gln	
	420	425	430
ctc ctc cag tcc aac	tgatggccca gatgcagcag	gaggccagag gacagagcag	1350
Leu Leu Gln Ser Asn			
	435		
aggatctttc caaccacatc tgctggctct ggggtcccag tgaattctgg tggcaaatat 1410			
atattttcac taactcaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaagg 1470			

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1489

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 35 40 45

Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser Asp Asn
 50 55 60

Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly Leu Leu
 65 70 75 80

Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp Ile Arg
 85 90 95

Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu Gly Lys
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Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly Leu Ser
 115 120 125

His Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr Gly Pro Val Arg Val
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Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn Ser Val
 145 150 155 160

Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His Arg Met
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Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp Asp Leu
 180 185 190

Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile Tyr Met
 195 200 205

Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys Lys Gln
 210 215 220

Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu Leu Thr
 225 230 235 240

Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val Gly Gln
 245 250 255

Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Ser Phe Ile

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260	265	270
Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu Thr Val		
275	280	285
Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu Pro Leu		
290	295	300
Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr Tyr Thr		
305	310	315
Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln Lys Lys		
	325	330
Ala Ile Ser Val Leu Glu Met Glu Asn Gly Tyr Trp Trp Cys Arg Lys		
	340	345
Lys Gln Arg Ala Gly Val Met Leu Thr Val Gly Thr Lys Pro Asp Gly		
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Ser Pro Asp Glu Arg Trp Cys Phe Arg Val Glu Glu Val Asn Trp Ala		
	370	375
Ser Trp Glu Gln Thr Leu Pro Thr Leu Cys Glu Asp Pro Ser Gly Ala		
385	390	395
Gly Val Pro Arg Thr Leu Glu Asn Pro Val Leu Ala Ser Pro Pro Lys		
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Leu Gln Ser Asn		
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cta ccc ctc tct ttg gcc gct tgc acc aag cag tgg gat gtg gta agc		96
Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val Val Ser		
	20	25
tac ctc ctg gag aac cca cac cag ccc gcc agc ctg cag gcc act gac		144
Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala Thr Asp		
	35	40

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tcc cag ggc aac aca gtc ctg cat gcc cta gtg atg atc tcg gac aac	192
Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser Asp Asn	
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tca gct gag aac att gca ctg gtg acc agc atg tat gat ggg ctc ctc	240
Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly Leu Leu	
65 70 75 80	
caa gct ggg gcc cgc ctc tgc cct acc gtg cag ctt gag gac atc cgc	288
Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp Ile Arg	
85 90 95	
aac ctg cag gat ctc acg cct ctg aag ctg gcc gcc aag gag ggc aag	336
Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu Gly Lys	
100 105 110	
atc gag att ttc agg cac atc ctg cag cgg gag ttt tca gga ctg agc	384
Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly Leu Ser	
115 120 125	
cac ctt tcc cga aag ttc acc gag tgg tgc tat ggg cct gtc cgg gtg	432
His Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr Gly Pro Val Arg Val	
130 135 140	
tcg ctg tat gac ctg gct tct gtg gac agc tgt gag gag aac tca gtg	480
Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn Ser Val	
145 150 155 160	
ctg gag atc att gcc ttt cat tgc aag agc ccg cac cga cac cga atg	528
Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His Arg Met	
165 170 175	
gtc gtt ttg gag ccc ctg aac aaa ctg ctg cag gcg aaa tgg gat ctg	576
Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp Asp Leu	
180 185 190	
ctc atc ccc aag ttc ttc tta aac ttc ctg tgt aat ctg atc tac atg	624
Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile Tyr Met	
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ttc atc ttc acc gct gtt gcc tac cat cag cct acc ctg aag aag cag	672
Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys Lys Gln	
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Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu Leu Thr	
225 230 235 240	
ggc cac atc ctt atc ctg cta ggg ggg atc tac ctc ctc gtg ggc cag	768
Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val Gly Gln	
245 250 255	
ctg tgg tac ttc tgg cgg cgc cac gtg ttc atc tgg atc tcg ttc ata	816
Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Phe Ile	
260 265 270	
gac agc tac ttt gaa atc ctc ttc ctg ttc cag gcc ctg ctc aca gtg	864
Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu Thr Val	

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275	280	285	
gtg tcc cag gtg ctg tgt ttc ctg gcc atc gag tgg tac ctg ccc ctg			912
Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu Pro Leu			
290	295	300	
ctt gtg tct gcg ctg gtg ctg ggc tgg ctg aac ctg ctt tac tat aca			960
Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr Tyr Thr			
305	310	315	320
cgt ggc ttc cag cac aca ggc atc tac agt gtc atg atc cag aag aaa			1008
Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln Lys Lys			
	325	330	335
gcc atc tct gtc ctg gag atg gag aat ggc tat tgg tgg tgc agg aag			1056
Ala Ile Ser Val Leu Glu Met Glu Asn Gly Tyr Trp Trp Cys Arg Lys			
	340	345	350
aag cag cgg gca ggt gtg atg ctg acc gtt ggc act aag cca gat ggc			1104
Lys Gln Arg Ala Gly Val Met Leu Thr Val Gly Thr Lys Pro Asp Gly			
	355	360	365
agc ccg gat gag cgc tgg tgc ttc agg gtg gag gag gtg aac tgg gct			1152
Ser Pro Asp Glu Arg Trp Cys Phe Arg Val Glu Glu Val Asn Trp Ala			
	370	375	380
tca tgg gag cag acg ctg cct acg ctg tgt gag gac ccg tca ggg gca			1200
Ser Trp Glu Gln Thr Leu Pro Thr Leu Cys Glu Asp Pro Ser Gly Ala			
385	390	395	400
ggt gtc cct cga act ctc gag aac cct gtc ctg gct tcc cct ccc aag			1248
Gly Val Pro Arg Thr Leu Glu Asn Pro Val Leu Ala Ser Pro Pro Lys			
	405	410	415
gag gat gag gat ggt gcc tct gag gaa aac tat gtg ccc gtc cag ctc			1296
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Leu Gln Ser Asn			
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<222> (2)..(1663)

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gat gtg gtg acc tac ctc ctg gag aac cca cac cag ccg gcc agc ctg 97
Asp Val Val Thr Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu
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gag gcc acc gac tcc ctg ggc aac aca gtc ctg cat gct ctg gta atg 145
Glu Ala Thr Asp Ser Leu Gly Asn Thr Val Leu His Ala Leu Val Met
    35             40             45

att gca gat aac tcg cct gag aac agt gcc ctg gtg atc cac atg tac 193
Ile Ala Asp Asn Ser Pro Glu Asn Ser Ala Leu Val Ile His Met Tyr
    50             55             60

gac ggg ctt cta caa atg ggg gcg cgc ctc tgc ccc act gtg cag ctt 241
Asp Gly Leu Leu Gln Met Gly Ala Arg Leu Cys Pro Thr Val Gln Leu
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gag gaa atc tcc aac cac caa ggc ctc aca ccc ctg aaa cta gcc gcc 289
Glu Glu Ile Ser Asn His Gln Gly Leu Thr Pro Leu Lys Leu Ala Ala
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aag gaa ggc aaa atc gag att ttc agg cac att ctg cag cgg gaa ttc 337
Lys Glu Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe
   100             105             110

tca gga ccg tac cag ccc ctt tcc cga aag ttt act gag tgg tgt tac 385
Ser Gly Pro Tyr Gln Pro Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr
   115             120             125

ggg cct gtg cgg gta tcg ctg tac gac ctg tcc tct gtg gac agc tgg 433
Gly Pro Val Arg Val Ser Leu Tyr Asp Leu Ser Ser Val Asp Ser Trp
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gaa aag aac tcg gtg ctg gag atc atc gct ttt cat tgc aag agc ccg 481
Glu Lys Asn Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro
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aac cgg cac cgc atg gtg gtt tta gaa cca ctg aac aag ctt ctg cag 529
Asn Arg His Arg Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln
   165             170             175

gag aaa tgg gat cgg ctc gtc tca aga ttc ttc ttc aac ttc gcc tgc 577
Glu Lys Trp Asp Arg Leu Val Ser Arg Phe Phe Phe Asn Phe Ala Cys
   180             185             190

tac ttg gtc tac atg ttc atc ttc acc gtc gtt gcc tac cac cag cct 625
Tyr Leu Val Tyr Met Phe Ile Phe Thr Val Val Ala Tyr His Gln Pro
   195             200             205

tcc ctg gat cag cca gcc atc ccc tca tca aaa gcg act ttt ggg gaa 673

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Ser	Leu	Asp	Gln	Pro	Ala	Ile	Pro	Ser	Ser	Lys	Ala	Thr	Phe	Gly	Glu		
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Ser	Met	Leu	Leu	Leu	Gly	His	Ile	Leu	Ile	Leu	Leu	Gly	Gly	Ile	Tyr		
225					230					235					240		
ctc	tta	ctg	ggc	cag	ctg	tgg	tac	ttt	tgg	cgg	cgg	cgc	ctg	ttt	atc	769	
Leu	Leu	Leu	Gly	Gln	Leu	Trp	Tyr	Phe	Trp	Arg	Arg	Arg	Leu	Phe	Ile		
				245					250					255			
tgg	atc	tca	ttc	atg	gac	agc	tac	ttt	gaa	atc	ctc	ttt	ctc	ctt	cag	817	
Trp	Ile	Ser	Phe	Met	Asp	Ser	Tyr	Phe	Glu	Ile	Leu	Phe	Leu	Leu	Gln		
			260					265					270				
gct	ctg	ctc	aca	gtg	ctg	tcc	cag	gtg	ctg	cgc	ttc	atg	gag	act	gaa	865	
Ala	Leu	Leu	Thr	Val	Leu	Ser	Gln	Val	Leu	Arg	Phe	Met	Glu	Thr	Glu		
			275				280					285					
tgg	tac	cta	ccc	ctg	cta	gtg	tta	tcc	cta	gtg	ctg	ggc	tgg	ctg	aac	913	
Trp	Tyr	Leu	Pro	Leu	Leu	Val	Leu	Ser	Leu	Val	Leu	Gly	Trp	Leu	Asn		
	290					295					300						
ctg	ctt	tac	tac	aca	cgg	ggc	ttt	cag	cac	aca	ggc	atc	tac	agt	gtc	961	
Leu	Leu	Tyr	Tyr	Thr	Arg	Gly	Phe	Gln	His	Thr	Gly	Ile	Tyr	Ser	Val		
305					310					315					320		
atg	atc	cag	aag	gtc	atc	ctt	cga	gac	ctg	ctc	cgt	ttc	ctg	ctg	gtc	1009	
Met	Ile	Gln	Lys	Val	Ile	Leu	Arg	Asp	Leu	Leu	Arg	Phe	Leu	Leu	Val		
				325					330				335				
tac	ctg	gtc	ttc	ctt	ttc	ggc	ttt	gct	gta	gcc	cta	gta	agc	ttg	agc	1057	
Tyr	Leu	Val	Phe	Leu	Phe	Gly	Phe	Ala	Val	Ala	Leu	Val	Ser	Leu	Ser		
			340					345					350				
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Arg	Glu	Ala	Arg	Ser	Pro	Lys	Ala	Pro	Glu	Asp	Asn	Asn	Ser	Thr	Val		
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acg	gaa	cag	ccc	acg	gtg	ggc	cag	gag	gag	gag	cca	gct	cca	tat	cgg	1153	
Thr	Glu	Gln	Pro	Thr	Val	Gly	Gln	Glu	Glu	Glu	Pro	Ala	Pro	Tyr	Arg		
		370				375					380						
agc	att	ctg	gat	gcc	tcc	cta	gag	ctg	ttc	aag	ttc	acc	att	ggg	atg	1201	
Ser	Ile	Leu	Asp	Ala	Ser	Leu	Glu	Leu	Phe	Lys	Phe	Thr	Ile	Gly	Met		
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ggg	gag	ctg	gct	ttc	cag	gaa	cag	ctg	cgt	ttt	cgt	ggg	gtg	gtc	ctg	1249	
Gly	Glu	Leu	Ala	Phe	Gln	Glu	Gln	Leu	Arg	Phe	Arg	Gly	Val	Val	Leu		
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ctg	ttg	ctg	ttg	gcc	tac	gtc	ctt	ctc	acc	tac	gtc	ctg	ctg	ctc	aac	1297	
Leu	Leu	Leu	Leu	Ala	Tyr	Val	Leu	Leu	Thr	Tyr	Val	Leu	Leu	Leu	Asn		
				420				425					430				
atg	ctc	att	gct	ctc	atg	agc	gaa	act	gtc	aac	cac	gtt	gct	gac	aac	1345	
Met	Leu	Ile	Ala	Leu	Met	Ser	Glu	Thr	Val	Asn	His	Val	Ala	Asp	Asn		
			435				440					445					

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gag aat ggt tac tgg tgg tgc cgg agg aag aaa cat cgt gaa ggg agg 1441
 Glu Asn Gly Tyr Trp Trp Cys Arg Arg Lys Lys His Arg Glu Gly Arg
 465 470 475 480

ctg ctg aaa gtc ggc acc agg ggg gat ggt acc cct gat gag cgc tgg 1489
 Leu Leu Lys Val Gly Thr Arg Gly Asp Gly Thr Pro Asp Glu Arg Trp
 485 490 495

tgc ttc agg gtg gag gaa gta aat tgg gct gct tgg gag aag act ctt 1537
 Cys Phe Arg Val Glu Glu Val Asn Trp Ala Ala Trp Glu Lys Thr Leu
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ccc acc tta tct gag gat cca tca ggg cca ggc atc act ggt aat aaa 1585
 Pro Thr Leu Ser Glu Asp Pro Ser Gly Pro Gly Ile Thr Gly Asn Lys
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aag aac cca acc tct aaa ccg ggg aag aac agt gcc tca gag gaa gac 1633
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 35 40 45

Ile Ala Asp Asn Ser Pro Glu Asn Ser Ala Leu Val Ile His Met Tyr
 50 55 60

Asp Gly Leu Leu Gln Met Gly Ala Arg Leu Cys Pro Thr Val Gln Leu
 65 70 75 80

Glu Glu Ile Ser Asn His Gln Gly Leu Thr Pro Leu Lys Leu Ala Ala
 85 90 95

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Lys Glu Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe
 100 105 110
 Ser Gly Pro Tyr Gln Pro Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr
 115 120 125
 Gly Pro Val Arg Val Ser Leu Tyr Asp Leu Ser Ser Val Asp Ser Trp
 130 135 140
 Glu Lys Asn Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro
 145 150 155 160
 Asn Arg His Arg Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln
 165 170 175
 Glu Lys Trp Asp Arg Leu Val Ser Arg Phe Phe Phe Asn Phe Ala Cys
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 Tyr Leu Val Tyr Met Phe Ile Phe Thr Val Val Ala Tyr His Gln Pro
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 210 215 220
 Ser Met Leu Leu Leu Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr
 225 230 235 240
 Leu Leu Leu Gly Gln Leu Trp Tyr Phe Trp Arg Arg Arg Leu Phe Ile
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 Trp Ile Ser Phe Met Asp Ser Tyr Phe Glu Ile Leu Phe Leu Leu Gln
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 275 280 285
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 Met Ile Gln Lys Val Ile Leu Arg Asp Leu Leu Arg Phe Leu Leu Val
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 Arg Glu Ala Arg Ser Pro Lys Ala Pro Glu Asp Asn Asn Ser Thr Val
 355 360 365
 Thr Glu Gln Pro Thr Val Gly Gln Glu Glu Glu Pro Ala Pro Tyr Arg
 370 375 380
 Ser Ile Leu Asp Ala Ser Leu Glu Leu Phe Lys Phe Thr Ile Gly Met
 385 390 395 400
 Gly Glu Leu Ala Phe Gln Glu Gln Leu Arg Phe Arg Gly Val Val Leu
 405 410 415

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Leu Leu Leu Leu Ala Tyr Val Leu Leu Thr Tyr Val Leu Leu Leu Asn
 420 425 430
 Met Leu Ile Ala Leu Met Ser Glu Thr Val Asn His Val Ala Asp Asn
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 Ser Trp Ser Ile Trp Lys Leu Gln Lys Ala Ile Ser Val Leu Glu Met
 450 455 460
 Glu Asn Gly Tyr Trp Trp Cys Arg Arg Lys Lys His Arg Glu Gly Arg
 465 470 475 480
 Leu Leu Lys Val Gly Thr Arg Gly Asp Gly Thr Pro Asp Glu Arg Trp
 485 490 495
 Cys Phe Arg Val Glu Glu Val Asn Trp Ala Ala Trp Glu Lys Thr Leu
 500 505 510
 Pro Thr Leu Ser Glu Asp Pro Ser Gly Pro Gly Ile Thr Gly Asn Lys
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 Lys Asn Pro Thr Ser Lys Pro Gly Lys Asn Ser Ala Ser Glu Glu Asp
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 His Leu Pro Leu Gln Val Leu Gln Ser Pro
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 Asp Val Val Thr Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu
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 gag gcc acc gac tcc ctg ggc aac aca gtc ctg cat gct ctg gta atg 144
 Glu Ala Thr Asp Ser Leu Gly Asn Thr Val Leu His Ala Leu Val Met
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 att gca gat aac tcg cct gag aac agt gcc ctg gtg atc cac atg tac 192
 Ile Ala Asp Asn Ser Pro Glu Asn Ser Ala Leu Val Ile His Met Tyr
 50 55 60
 gac ggg ctt cta caa atg ggg gcg cgc ctc tgc ccc act gtg cag ctt 240
 Asp Gly Leu Leu Gln Met Gly Ala Arg Leu Cys Pro Thr Val Gln Leu
 65 70 75 80

- 34 -

gag gaa atc tcc aac cac caa ggc ctc aca ccc ctg aaa cta gcc gcc	288
Glu Glu Ile Ser Asn His Gln Gly Leu Thr Pro Leu Lys Leu Ala Ala	
85 90 95	
aag gaa ggc aaa atc gag att ttc agg cac att ctg cag cgg gaa ttc	336
Lys Glu Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe	
100 105 110	
tca gga ccg tac cag ccc ctt tcc cga aag ttt act gag tgg tgt tac	384
Ser Gly Pro Tyr Gln Pro Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr	
115 120 125	
ggt cct gtg cgg gta tcg ctg tac gac ctg tcc tct gtg gac agc tgg	432
Gly Pro Val Arg Val Ser Leu Tyr Asp Leu Ser Ser Val Asp Ser Trp	
130 135 140	
gaa aag aac tcg gtg ctg gag atc atc gct ttt cat tgc aag agc ccg	480
Glu Lys Asn Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro	
145 150 155 160	
aac cgg cac cgc atg gtg gtt tta gaa cca ctg aac aag ctt ctg cag	528
Asn Arg His Arg Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln	
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Glu Lys Trp Asp Arg Leu Val Ser Arg Phe Phe Phe Asn Phe Ala Cys	
180 185 190	
tac ttg gtc tac atg ttc atc ttc acc gtc gtt gcc tac cac cag cct	624
Tyr Leu Val Tyr Met Phe Ile Phe Thr Val Val Ala Tyr His Gln Pro	
195 200 205	
tcc ctg gat cag cca gcc atc ccc tca tca aaa gcg act ttt ggg gaa	672
Ser Leu Asp Gln Pro Ala Ile Pro Ser Ser Lys Ala Thr Phe Gly Glu	
210 215 220	
tcc atg ctg ctg ctg ggc cac att ctg atc ctg ctt ggg ggt att tac	720
Ser Met Leu Leu Leu Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr	
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Leu Leu Leu Gly Gln Leu Trp Tyr Phe Trp Arg Arg Arg Leu Phe Ile	
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Trp Ile Ser Phe Met Asp Ser Tyr Phe Glu Ile Leu Phe Leu Leu Gln	
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Ala Leu Leu Thr Val Leu Ser Gln Val Leu Arg Phe Met Glu Thr Glu	
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Trp Tyr Leu Pro Leu Leu Val Leu Ser Leu Val Leu Gly Trp Leu Asn	
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Leu Leu Tyr Tyr Thr Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val	
305 310 315 320	

- 35 -

atg atc cag aag gtc atc ctt cga gac ctg ctc cgt ttc ctg ctg gtc	1008
Met Ile Gln Lys Val Ile Leu Arg Asp Leu Leu Arg Phe Leu Leu Val	
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Tyr Leu Val Phe Leu Phe Gly Phe Ala Val Ala Leu Val Ser Leu Ser	
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aga gag gcc cga agt ccc aaa gcc cct gaa gat aac aac tcc aca gtg	1104
Arg Glu Ala Arg Ser Pro Lys Ala Pro Glu Asp Asn Asn Ser Thr Val	
355 360 365	
acg gaa cag ccc acg gtg ggc cag gag gag gag cca gct cca tat cgg	1152
Thr Glu Gln Pro Thr Val Gly Gln Glu Glu Glu Pro Ala Pro Tyr Arg	
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Ser Ile Leu Asp Ala Ser Leu Glu Leu Phe Lys Phe Thr Ile Gly Met	
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Gly Glu Leu Ala Phe Gln Glu Gln Leu Arg Phe Arg Gly Val Val Leu	
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Leu Leu Leu Leu Ala Tyr Val Leu Leu Thr Tyr Val Leu Leu Leu Asn	
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Met Leu Ile Ala Leu Met Ser Glu Thr Val Asn His Val Ala Asp Asn	
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Ser Trp Ser Ile Trp Lys Leu Gln Lys Ala Ile Ser Val Leu Glu Met	
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Leu Leu Lys Val Gly Thr Arg Gly Asp Gly Thr Pro Asp Glu Arg Trp	
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Cys Phe Arg Val Glu Glu Val Asn Trp Ala Ala Trp Glu Lys Thr Leu	
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Pro Thr Leu Ser Glu Asp Pro Ser Gly Pro Gly Ile Thr Gly Asn Lys	
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Lys Asn Pro Thr Ser Lys Pro Gly Lys Asn Ser Ala Ser Glu Glu Asp	
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545

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<211> 16

<212> PRT

<213> Artificial Sequence

<220>

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<223> Description of Artificial Sequence: synthetic
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<220>

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peptide

<400> 15

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<210> 16

<211> 20

- 37 -

<212> DNA

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<223> Description of Artificial Sequence: primer

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<212> DNA

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<223> Description of Artificial Sequence:primer

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<213> Artificial Sequence

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<223> Description of Artificial Sequence:primer

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<211> 630

<212> PRT

<213> Homo sapiens

<400> 20

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			20					25					30		

- 38 -

Gly Ser Gly Leu Pro Pro Met Glu Ser Gln Phe Gln Gly Glu Asp Arg
 35 40 45
 Lys Phe Ala Pro Gln Ile Arg Val Asn Leu Asn Tyr Arg Lys Gly Thr
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 Gly Ala Ser Gln Pro Asp Pro Asn Arg Phe Asp Arg Asp Arg Leu Phe
 65 70 75 80
 Asn Ala Val Ser Arg Gly Val Pro Glu Asp Leu Ala Gly Leu Pro Glu
 85 90 95
 Tyr Leu Ser Lys Thr Ser Lys Tyr Leu Thr Asp Ser Glu Tyr Thr Glu
 100 105 110
 Gly Ser Thr Gly Lys Thr Cys Leu Met Lys Ala Val Leu Asn Leu Lys
 115 120 125
 Asp Gly Val Asn Ala Cys Ile Leu Pro Leu Leu Gln Ile Asp Arg Asp
 130 135 140
 Ser Gly Asn Pro Gln Pro Leu Val Asn Ala Gln Cys Thr Asp Asp Tyr
 145 150 155 160
 Tyr Arg Gly His Ser Ala Leu His Ile Ala Ile Glu Lys Arg Ser Leu
 165 170 175
 Gln Cys Val Lys Leu Leu Val Glu Asn Gly Ala Asn Val His Ala Arg
 180 185 190
 Ala Cys Gly Arg Phe Phe Gln Lys Gly Gln Gly Thr Cys Phe Tyr Phe
 195 200 205
 Gly Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val
 210 215 220
 Val Ser Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala
 225 230 235 240
 Thr Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser
 245 250 255
 Asp Asn Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly
 260 265 270
 Leu Leu Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp
 275 280 285
 Ile Arg Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu
 290 295 300
 Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly
 305 310 315 320
 Leu Ser His Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr Gly Pro Val
 325 330 335

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Arg Val Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn
 340 345 350
 Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His
 355 360 365
 Arg Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp
 370 375 380
 Asp Leu Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile
 385 390 395 400
 Tyr Met Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys
 405 410 415
 Lys Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu
 420 425 430
 Leu Thr Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val
 435 440 445
 Gly Gln Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Ser
 450 455 460
 Phe Ile Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu
 465 470 475 480
 Thr Val Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu
 485 490 495
 Pro Leu Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr
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 Tyr Thr Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln
 515 520 525
 Lys Lys Ala Ile Ser Val Leu Glu Met Glu Asn Gly Tyr Trp Trp Cys
 530 535 540
 Arg Lys Lys Gln Arg Ala Gly Val Met Leu Thr Val Gly Thr Lys Pro
 545 550 555 560
 Asp Gly Ser Pro Asp Glu Arg Trp Cys Phe Arg Val Glu Glu Val Asn
 565 570 575
 Trp Ala Ser Trp Glu Gln Thr Leu Pro Thr Leu Cys Glu Asp Pro Ser
 580 585 590
 Gly Ala Gly Val Pro Arg Thr Leu Glu Asn Pro Val Leu Ala Ser Pro
 595 600 605
 Pro Lys Glu Asp Glu Asp Gly Ala Ser Glu Glu Asn Tyr Val Pro Val
 610 615 620
 Gln Leu Leu Gln Ser Asn
 625 630

INTERNATIONAL SEARCH REPORT

Intern ational Application No

PCT/US 99/26701

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N5/10 C07K14/705 C07K16/28 G01N33/53
C12Q1/68 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K G01N C12Q A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CATERINA M J ET AL: "THE CAPSAICIN RECEPTOR: A HEAT-ACTIVATED ION CHANNEL IN THE PAIN PATHWAY" NATURE, GB, MACMILLAN JOURNALS LTD. LONDON, vol. 389, 23 October 1997 (1997-10-23), pages 816-824, XP002075020 ISSN: 0028-0836 cited in the application abstract figure 5 page 821, left-hand column, paragraph 2	1-26
P, X	WO 99 09140 A (BRAKE ANTHONY ; JULIUS DAVID J (US); UNIV CALIFORNIA (US); CATERINA) 25 February 1999 (1999-02-25) abstract 100% identity in 1717 BP overlap between SEQ ID NO 3 of WO9909140 and SEQ ID NO 10 ----- -/-	1-26

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 April 2000

Date of mailing of the international search report

10/05/2000

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Authorized officer

Lejeune, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/26701

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 37675 A (BRAKE ANTHONY J ;JULIUS DAVID J (US); UNIV CALIFORNIA (US); CATERI) 29 July 1999 (1999-07-29) abstract 99.7% identity in 2533 BP overlap between SEQ ID NO 33 of W09937675 and SEQ ID NO 1 100% identity in 2380 BP overlap between SEQ ID NO 35 of W09937675 and SEQ ID NO 4 100% identity in 1717 BP overlap between SEQ ID NO 3 of W09937675 and SEQ ID NO 10	1-26
P,X	WO 99 37765 A (SMITHKLINE BEECHAM PLC) 29 July 1999 (1999-07-29) abstract 99.9% identity in 2351 BP overlap between SEQ ID NO 1 and 5 of W09937765 and SEQ ID NO 4	1-26
P,X	EP 0 943 683 A (SMITHKLINE BEECHAM PLC) 22 September 1999 (1999-09-22) abstract 99.6% identity in 2818 BP overlap between SEQ ID NO 1 of EP943683 and SEQ ID NO 1	1-26
P,X	EP 0 953 638 A (SYNTHELABO) 3 November 1999 (1999-11-03) abstract 99.8% identity in 2786 BP overlap between SEQ ID NO 1 of EP953638 and SEQ ID NO 4	1-26
P,X	CATERINA ET AL: "A capsaicin-receptor homologue with a high threshold for noxious heat" NATURE,GB,MACMILLAN JOURNALS LTD. LONDON, vol. 398, no. 398, 1 April 1999 (1999-04-01), pages 436-441-441, XP002105951 ISSN: 0028-0836 the whole document 99.8% identity in 2401 BP overlap between the sequence of hVRL and SEQ ID NO 4 100% identity in 1752 BP overlap between the sequence of rVRL and SEQ ID NO 10	1-26
X	WO 98 39448 A (HUMAN GENOME SCI INC) 11 September 1998 (1998-09-11) abstract page 112 page 163 99.3% identity in 1947 BP overlap between SEQ ID NO 191 of W09839448 and SEQ ID NO 4 98.1% identity in 1709 BP overlap between SEQ ID NO 307 of W09839448 and SEQ ID NO 4	1-11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/ 26701

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
**Remark: Although claims 24-26
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.**
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ N protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

Intern ial Application No
PCT/US 99/26701

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9909140	A	25-02-1999	AU 9115698 A AU 2466799 A WO 9937675 A	08-03-1999 09-08-1999 29-07-1999
WO 9937675	A	29-07-1999	AU 2466799 A AU 9115698 A WO 9909140 A	09-08-1999 08-03-1999 25-02-1999
WO 9937765	A	29-07-1999	NONE	
EP 0943683	A	22-09-1999	JP 11279196 A	12-10-1999
EP 0953638	A	03-11-1999	AU 2932799 A WO 9946377 A	27-09-1999 16-09-1999
WO 9839448	A		NONE	

